GREEN PIGMENTATION IN NEOTROPICAL FROGS

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
December, 1967

ACKNOWLEDGMENTS

I wish to thank Dr. Coleman J. Goin for suggesting the challenging topic of this paper and for his continued interest and support during the various phases of its study. Dr. Goin has aided in the identification of frogs used in this study and has extended many other courtesies which are deeply appreciated. I am grateful to Dr. Mildred Griffith, Dr. Frank J. S. Maturo and Dr. James Nation for their aid during this study and for suggestions which improved the dissertation. To Olive B. Goin, I owe thanks for many kind acts and stimulating discussions.

Several individuals have made laboratory and field facilities available to me. Among these were: Dr. James Nation, Dr. Ward Noyes, Dr. Albert Laessle, Dr. Frank Nordlie, Dr. Paul Elliott, and Dr. James Gregg, all of the University of Florida; Dr. Harold Heatwole, University of Puerto Rico (El Yunke Biological Station); Mr. Bernard Lewis and Dr. Thomas Farr, Institute of Jamaica; Dr. Ivan Goodbody, University of West Indies, Kingston, Jamaica; Dr. Jocelyn Crane, Simla Biological Station, Trinidad; and Mr. Walter Polder, Paramaribo, Surinam.

To the following persons, I am grateful for the opportunity to study the specimens of their respective institutions: Dr. Ernest Williams, Museum of Comparative Zoology, Harvard University; Mr. C. M. Bogert and Dr. Richard Zweifel, American Museum of Natural History;

Dr. James Bohlke, Philadelphia Academy of Natural Sciences; Dr. Doris Cochran and Dr. James Peters, United States National Museum; Mr. Neil Richmond and Dr. Clarence McCoy, Carnegie Museum; Dr. Charles F. Walker, University of Michigan Museum of Zoology; Dr. Robert Inger, Chicago Natural History Museum; Dr. Walter Auffenberg, Florida State Museum; Mr. Bernard Lewis and Dr. Thomas Farr, Institute of Jamaica.

I am grateful to Dr. Thomas Goreau, University of the West Indies, Dr. Margaret Stewart, State University of New York at Albany, and others for enlightening discussions. Bonneye Greene, Thomas Quarles, Robert MacFarlane and John Anderson kindly rendered technical assistance. Forest service personnel in Jamaica and Surinam made it possible for me to visit several remote localities. I appreciate the aid rendered by members of the U. S. Consular Service. Grants for travel expenses were received from the University of Florida Graduate School, the Sigma Xi- RESA Fund and the National Science Foundation (GB-3644, to Dr. Coleman J. Goin).

The services of Rhea Warren, Stephen Bass, Murray de la Fuente and others who aided in the collection of specimens are appreciated greatly. Janet Bungay and Geraldine Lennon aided in the preparation of the manuscript. My wife, Dorothy, assisted in the histological portion of the study, in preparation of the manuscript, and in giving moral support.

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INTRODUCTION

A wide variety of colors is demonstrated by anurans, particularly those of tropical areas. Frogs and toads which spend most of their time on tree trunks, among the dead leaves of the forest floor, about rocks, or underground, are usually brown, gray or black due to the presence of melanin in melanophores. Red or yellow colors are usually due to lipid pigments in the lipophores, and white results from reflection of visible light by guanine crystals of guanophores. Green hues are often seen among anurans, especially in the skin of those closely associated with green vegetation.

Studies have shown that the green skin color of many frogs in life is the result of combined effects of three types of chromatophores located in the upper portion of the dermis. Lipophores containing yellow pigment are found just under the basement membrane of the epidermis, in close association with the guanophores immediately below them. Melanophores are found below the guanophores, but have processes extending into the guanophore layer. Visible light is diffracted as it impinges upon the guanine crystals of the guanophores; the blue and green portions of the spectrum tend to be reflected, while light of longer wave lengths is absorbed by the melanophores. As the light of shorter wave lengths passes back through the lipophores, the blue portion is absorbed and only green is reflected from the skin. Modification of lipophores following fixation of the frog causes the skin to appear

blue. Thus, the green color of frog skin of this type results from the physical arrangement of three pigments, none of which is green (Schmidt, 1919, 1920, 1921; Kawaguti, Kamishima and Sato, 1965; Elias, 1943).

Though most frogs with green skin appear to fit the pattern described, some tree frogs do not. Notable are some of the small hylids such as Hyla wilderi. This species has few melanophores and appears to be a diffuse green color throughout the body. When preserved in alcohol, a green pigment is extracted and the frog becomes whitish in color. In this species, as well as in others, there is no blue color which supplants the green upon preservation. Indications are that structural effects are not involved and that this type of green color in frogs is due solely to a green pigment or pigments. Reports of internal green pigment substantiate this idea.

This paper is concerned with three facets of the biology of green neotropical frogs: (1) identification of the green pigment or pigments; (2) ecology; and (3) physiology of green frogs, with emphasis upon possible factors influencing pigment accumulation. Since these three areas require different methods of study and yield various types of information, they will be presented in separate sections.

LITERATURE REVIEW

Naturally Occurring Green Pigments

Porphyrin compounds and their derivatives constitute the vast majority of green pigments among living organisms. Among the best known of these are the chlorophylls, green hemoglobins and green bile pigments. Others include the chlorocruorin blood pigments of polychaetes; turacoverdin from the feathers of plantain-eating touracos (Fox, 1953); myeloperoxidase, the green enzyme of white blood cells and certain tumors (Agner, 1941); and verdohemochromes, which are intermediate compounds that appear during the degradation of heme compounds to bile pigments in vitro (Lemberg and Legge, 1949).

Some tunicates have a pale green pigment which contains vanadium. The structure of this pigment is not well known, but it may be similar to some of the bile pigments. Green pigments of crustacean hypodermis and eggs result from conjugation of a reddish carotene pigment with proteins (Fox, 1953).

Green pigments of frogs

In 1850, Kunde wrote the first paper concerning a green pigment in frogs (see Lemberg and Legge, 1949, p. 506). He observed biliverdin in frogs' blood serum after liver extirpation. Lemberg (1935), Nisimaru (1931), Cabello (1943), Rodriguez Garay, Noir and Royer (1965), and Barrio (1965[a]) indicated that amphibian bile contains biliverdin as

its bile pigment. Lester and Schmid (1961) found biliverdin in some samples of frog bile, but believed bilirubin to be the main bile pigment of anurans. A claim was made by von Recklinghausen (1883) that formation of biliverdin takes place in sterile frog blood. Rich (1925) indicated that this had not been confirmed. Cabello (1943) caused the formation of green serum in Bufo arenarum by administration of phenylhydrazine and ligation of the bile duct. Rodriguez Garay et al. (1965) found biliverdin glucuronide in the bile of Bufo arenarum.

The first report of externally visible green pigment in frogs appears to be the one by Peters (1873), who indicated that the skeleton of Pseudis minuta was green. Camerano (1879) believed the green bones of Pseudis paradoxus to be due to the presence of ferrous phosphate. The recorder of the Zoological Record (Boulenger, 1880) took exception to Peters' paper by stating that Pseudis minuta does not have green bones. However, Boulenger (1883) took note of the green eggs of pseudids. Fernandez and Fernandez (1921), Miranda-Ribeiro (1926), Parker (1935), and Gallardo (1961) also noted green eggs or tissues in this family.

In 1910, Podiapolsky described a "chlorophyll" pigment from Hyla arborea and Rana esculenta, two European species. It may be noted here that reports by Schmidt (1919-1921) upon these two species, and by Kawaguti, Kamishima and Sato (1965) upon Hyla arborea, do not support this. More recently, Dunn (1926), A. Lutz (1924, 1938), B. Lutz (1948, 1954), Cochran (1955), Lynn (1958), and Bokermann (1964) have noted green coloration of epithelia, muscles and bones in certain

hylid species. Dunn (1931), B. Lutz (1947), and Savage (1967) noted the presence of green bones in certain species of centrolenids.

Barrio (1965a), working with frogs from Argentina, found that five of twelve hylid species and all three of the pseudid species which he studied had green tissues. Individuals of twenty-two species of four other families lacked the green pigment in their tissues. The species that had green tissues were similar in that the muscular and subcutaneous tissue, lymph, walls of the digestive tract, eggs of mature females, and especially the bones were strongly pigmented. He attributed this situation to high concentrations of biliverdin in the blood. The highest concentration, 11.9 mg/100ml, was found in the serum of Hyla punctata. In addition, he pointed out that biliverdin is decomposed by formalin. This point clarified an earlier controversy (Boulenger, 1880) and also indicated a reason for so little attention being given to this phenomenon.

Another paper by Barrio (1965b) brought out the fact that populations of Hyla pulchella differ greatly in their serum concentrations of biliverdin; two of the five subspecies lack the pigment altogether.

Green tissues of other animals

Green tissues are not limited to frogs. Peters (1873) noted that the green bones of Pseudis minuta were similar in color to those of the marine garfish Belone. The relatively recent papers of Wagenaar (1939), Fontaine (1941a, 1941b), Willstaedt (1941), Caglar (1945), and Fox (1953) concerning green bones of teleost fish pointed toward

biliverdin or other hematin derivatives as the green pigment. Lonnberg (1934) noted green color in tissues of the African lungfish, Protopterus annectens. Lemberg and Legge (1949, p. 506, pp. 569-570) listed other vertebrate structures and invertebrates which contain biliverdin.

Identification of the green pigment

Barrio (1965[a]) has amply demonstrated by chemical and spectrophotometric means that biliverdin is primarily responsible for the green color of tissues in the frogs which he studied. Through electrophoretic fractionation of green serum, he found that most of the biliverdin was combined with globulin proteins and a lesser amount was associated with albumin. Lester and Schmid (1961) have shown that adult anurans have an enzyme for conjugating bile pigments and Rodriguez Garay, Noir and Royer (1965) have found small quantities of water soluble biliverdin glucuronide in Bufo arenarum. Barrio (1965[a]) considered the biliverdin to be unconjugated, since it migrated at the same rate as free biliverdin on paper chromatographs, was completely soluble in chloroform, and insoluble in water.

Characteristics of Biliverdin

Chemical properties and reactions

Biliverdin ($C_{33}H_{34}O_6N_4$) is composed of four pyrrole groups linked together by three methyne bridges. Its structural formula may be drawn in the form of a chain, but is more precisely represented by the incomplete ring form. There is an obvious similarity of biliverdin to two of its precursors, protoporphyrin IXa and heme of hemoglobin. It differs from them in that its ring structure is incomplete; also,

it does not contain iron as does heme. Mesobiliverdin is formed from biliverdin by reduction of the vinyl side chains to ethyl groups.

Chemical reactions of biliverdin include the Gmelin reaction. Addition of fuming nitric acid to tetrapyrrole bile pigments converts all initially to biliverdins. These are oxidized further to yield a succession of pigments from blue-green through violet, red and yellow to colorless compounds.

As already indicated, reduction of the vinyl side chains of biliverdin results in mesobiliverdin. Reduction of the middle methyne bridge yields bilirubin, a common bile pigment of mammals and other vertebrates. Bilirubin may be reduced further to other bile pigments.

Biliverdin is destroyed by heating with concentrated sulfuric acid, but mesobiliverdin is not. Stable hydrochlorides or hydrobromides result from treatment with the appropriate acid. Complex salts may be formed with iron, zinc and copper. Biliverdin also undergoes esterification. Unlike bilirubin, biliverdin does not couple with diazotized sulfanilic acid to give the diazo reaction (Lemberg and Legge, 1949).

Physical properties

Biliverdin is moderately soluble in ether, in which it has a greenish-blue color. It may be extracted from ether by 1 percent hydrochloric acid, in which it has a blue-green color. These deep colors result from the conjugation of double bonds throughout the length of the molecule. Biliverdin is also soluble in methanol (Lemberg and Legge, 1949) and chloroform, but is insoluble in water (Barrio, 1965a).

The absorption curve of biliverdin has been shown (Figure 1). Mesobiliverdin has a similar curve, but its absorption maxima lie about 10 millimicrons further from the infrared than do those of biliverdin. Neither of these compounds has an absorption peak between 400-440 millimicrons (Soret band), which is characteristic of compounds with the closed porphyrin ring (Holden and Lemberg, 1939).

Biological properties

The biological properties of biliverdin are related to its physical and chemical properties. Of considerable importance is its solubility in different solvents. The fact that Barrio (1965a) did not find biliverdin in nervous tissue, ocular fluids, or urine may be directly related to the insolubility of unconjugated biliverdin in water.

Insolubility of biliverdin in water presents a problem in regard to its transport within the body and its excretion. In mammals, as well as some birds and reptiles and Bufo arenarum, this problem is not so important because the liver conjugates variable amounts of the bile pigments (either bilirubin or biliverdin) with glucuronic acid to form water-soluble glucuronides (Rodriguez Garay et al., 1965). These investigators also found a sodium salt of biliverdin, free biliverdin, and a substance which had chromatographic characteristics of a complex of bile acid and sodium biliverdinate in Bufo arenarum. The electrophoretic studies of Barrio (1965a) indicate that in the circulatory system biliverdin is associated primarily with globulin proteins, but some is conjugated with albumin. Non-esterified fatty acids appear to

displace bile pigment from serum proteins in new-born infants (Melichar, Polacek and Novak, 1962). Assuming that this also occurs in frogs, one would expect that when biliverdin exceeds the carrying capacity of the serum proteins, it will be deposited in the non-fluid tissues, staining them with its color. Evidence of the staining by biliverdin is available in many frogs, particularly where the gall bladder lies adjacent to the stomach wall.

Sources of Biliverdin

Hemoglobin and other hemoproteins such as myoglobin and oxidizing enzymes constitute the main sources of bile pigments. Although most studies of bile pigment formation are concerned with the origin of bilirubin in mammals, we may consider the origins of biliverdin to be the same, since it is generally considered to be a precursor of bilirubin.

In a recent paper, Israels et al. (1966) proposed a scheme in which there are four components of human bilirubin. Each of these components is characterized by a peak concentration of radioactive bilirubin at a specific interval after the labeling of bilirubin precursors. The major bilirubin component is formed from hemoglobin of old red blood cells and makes its appearance about 100 days after the radioactive isotope is administered. The lesser components appear more rapidly; collectively, they are referred to as early bilirubin. One of these reaches a peak three to five days after ingestion of the radioactively labeled compound and is believed to originate from a heme loss

during erythropoiesis. The third component is formed during the first twenty-four hours, probably beginning during the second hour after administration. The fourth fraction appears most rapidly--within a few minutes of intake. The latter fraction was detected in rat liver homogenate after five minutes' incubation with C^{14} labeled delta-aminolevulinic acid (a precursor of protoporphyrin). Presence of such a component was suspected when the radioactive bilirubin recovered during the first two hours was found to account for one-third of the twenty-four-hour total. A block of the bile duct or similar obstruction is shown to increase the latter bilirubin fraction. Perfusion experiments and other data show that the latter two fractions are of hepatic rather than erythropoietic origin. The main non-erythropoietic component of bilirubin, which is relatively slow in being formed, "is probably related to the turnover of the major hemeproteins," such as liver catalase. The smaller component arises rapidly from a pool of heme-protein, heme, or heme precursors which has a high turnover rate. Barrio (1965[a]) suggested that high concentrations of biliverdin in frogs may be due to increased formation of early biliverdin.

Mechanisms of bile pigment formation

Earlier hypotheses. -- Much study has gone into the chemistry of bile pigments and their formation from hemoglobin. Most of these studies have dealt with non-enzymatic reactions of bile pigments and related compounds carried out in vitro. Early studies of hemoglobin degradation employed harsh techniques and detected such substances as hematin and hematoporphyrin. With such supposed intermediate compounds

as these, and the knowledge that globin and iron are separated from the tetrapyrrole, it was generally assumed that hemoglobin was degraded as follows: hemoglobin, hematin, hematoporphyrin, bilirubin, with biliverdin a secondary oxidation product of bilirubin (Lemberg and Legge, 1949). Later, Lemberg and others (see Lemberg and Legge, 1949; Foulkes, Lemberg and Purdom, 1951) succeeded in finding a sequence of relatively mild reactions which degraded hemoglobin to biliverdin. Although these reactions were not well understood and yielded only 15 per cent of the biliverdin expected, workers in the field generally accepted Lemberg's series of reactions as a hypothetical model of hemoglobin degradation in cells.

Enzymatic degradation of hemoglobin. -- Recent studies of Nakajima et al. (1963) have demonstrated an enzymatic pathway which is capable of degrading hemoglobin to biliverdin. These workers have characterized an enzyme which oxidizes the alpha-methyne bridge of the heme group to form a possible precursor of biliverdin. Most of this work was done with the pyridine hemichrome rather than hemoglobins, but their study of substrates is of considerable interest. The enzyme did not act upon alkaline hematin or protoporphyrin IX, and only weakly upon hemoglobin (contains ferrous ion) and hemiglobin (contains ferric ion). However, reaction of the enzyme with a complex substrate of hemoglobin and haptoglobin (a plasma protein which combines with extracellular hemoglobin), produced 49 per cent of the theoretical yield of biliverdin. With the hemoglobin-haptoglobin complex as a substrate, there was a lag period of five minutes before any noticeable reaction took place. There

was no lag period when a substrate of hemiglobin-haptoglobin was used. In addition, the enzymatic activity was about 50 per cent higher with hemiglobin-haptoglobin or carboxyhemoglobin-haptoglobin than with hemoglobin-haptoglobin as a substrate. Thus, it would appear that hemiglobin-haptoglobin is the primary substrate of the enzyme and that the lag in degradation of the hemoglobin-haptoglobin represents the period during which hemoglobin is being changed into hemiglobin. The enzyme acts only in the presence of oxygen and is considered an oxidase, rather than a peroxidase. It is known as heme alpha-methenyl oxygenase and is found largely in the liver and kidney, being nearly absent from the spleen and bone marrow. The product of this enzymatic reaction is then acted upon by a second enzyme, heme alpha-methenyl formylase, to yield biliverdin, iron and formaldehyde.

An interesting observation which may be inserted at this point is that biliverdin conversion to bilirubin has been shown to be under enzymatic control in the laboratory rat (Lester et al. 1966).

Causes of Chlorosis

Barrio (1965a) noted that the three families of Neotropical frogs which include chlorotic species--Hylidae, Centrolenidae and Pseudidae--also have in common an intercalary cartilage between the ultimate and penultimate phalanges of the digits. He suggested that these two common factors indicate a close relationship among these families. However, the intercalary bone of pseudids is believed to represent an adaptation for swimming, similar to the situation in aquatic mammals (Goin and Goin, 1962a). Most recent writers have considered the presence

of intercalary cartilages in the several families of tree frogs as an example of parallelism (Goin, 1961; Griffiths, 1963; Lynch and Freeman, 1966). Goin and Goin (1962[a], p. 231) stated, "The extra joint thus provided allows the last phalanx, with its adhesive disc, to be placed flat against the surface regardless of the position of the foot--an obvious advantage to the climbing form."

No unusual conditions such as high rates of hemolysis were noted by Barrio (1965[a]). He suggested that the chlorotic conditions which he witnessed were due to formation of early bile pigment. Hemolytic conditions are not recorded for Amphibia, but might be expected during metamorphosis when tadpole hemoglobin is replaced by frog hemoglobin (McCutcheon, 1936). Varela and Sellares (1938) noted a rapid decrease in the red cell count of Bufo arenarum at the end of the breeding period, a change which indicates rapid hemolysis.

It appears that no satisfactory explanation of chlorosis has been published. However, presence of biliverdin in the chlorotic species studied by Barrio (1965[a]) implicates hemoproteins, particularly hemoglobin. Through hemoglobin one might expect involvement of various structures and functions related to gaseous exchange.

Respiratory Studies

Embryonic and larval respiratory structures

Considerable research has been done on respiration as one of the most important processes of living organisms. Much of it has been directed toward micro-organisms, the mammalian species, and animals

possessing unusual respiratory structures (Negus, 1965). The several types and combinations of respiratory organs shown by species of Amphibia have been the interest of many investigators of respiration. Most reports have been concerned with the structure of respiratory organs and surfaces, rates of oxygen uptake, and the role of the blood and circulatory system in gaseous transport. Some work has been done on larval amphibian respiration, especially on those species of salamanders and frogs commonly used in embryological and physiological research, despite their small size. There are few studies, however, of adaptations in relation to the ecological situations of larval or adult amphibians (Foxon, 1964).

Structure and function of respiratory and associated circulatory organs of amphibians in general have been reviewed by Noble (1931) and more recently by Goin and Goin (1962a) and Foxon (1964). The present survey will be concerned specifically with the respiration of amphibian tadpoles and similar aquatic forms, as well as with hemoglobin and red blood cells of amphibians generally.

As the amphibian embryo develops, gaseous exchange first occurs through the egg surface, later through the external body surfaces (including gills), and finally through lungs, if present (Foxon, 1964). Among the species of anurans, the respiratory surface decreases proportionately as the diameter of the egg increases, since the volume of the egg increases as a cubic function of the radius, while the area increases as a squared function. No examples of enlarged surface area due to folding or other changes in the egg membrane are known (Noble, 1931).

However, there are considerable differences in size of amphibian eggs. Panton (1952) noted that aquatic eggs of Hyla brunnea were only one-half millimeter in diameter, whereas those of another frog (probably Eleutherodactylus jamaicensis) that did not develop in water, were several millimeters in diameter.

The sites of egg deposition and tadpole development appear related to respiratory processes. Dickerson (1906) believed that the jelly membranes of amphibian eggs retain heat longer than the surrounding water. Experiments by Savage (1950), using the eggs of Rana temporaria, showed that the mean temperature difference between eggs with jelly envelopes and plain water was 0.63° C. Moore (1940) suggested that the compact jelly masses (up to 10 centimeters in diameter) of Rana sylvatica slow the diffusion of oxygen to the embryos, and that this becomes critical as the metabolic rate increases in response to a temperature of about 25° C. However, Savage (1950) pointed out that water moves freely between individual jelly capsules of Rana temporaria, thus requiring diffusion of oxygen through only a few millimeters rather than several centimeters of jelly. Algae associated with jelly membranes of Rana sylvatica (Dickerson, 1906), Rana aurora (Wright and Wright, 1949) and Rana temporaria (Savage, 1961) may affect the respiration of these frogs' eggs.

Goin and Goin (1962b) noted that amphibian eggs may be laid singly or in clusters and may be attached to submerged objects, floating or settled in water, carried by parents, or be laid on land or vegetation.

There are few indications of respiratory rates of embryos being correlated with these types or sites of egg deposition. Panton's observation (1952) that a set of large eggs, probably those of Eleutherodactylus jamaicensis, failed to develop when placed in water may indicate that embryos which develop terrestrially receive insufficient oxygen when subjected to the reduced oxygen tension of water. Noble (1931) suggested that large eggs undergoing rapid development require more oxygen than they can obtain in water when surrounded by the egg capsule.

As the embryos develop and hatch, other respiratory structures are formed and behavioral patterns related to respiration arise. Organs of external respiration in tadpoles are the skin, the internal surface of the operculum, external and internal gills, the vascularized surface of the food filtering apparatus, and the lungs.

The most evident respiratory structures to appear in about the time of hatching are the external gills. These may be small as in Hyla vasta or enlarged as in Hyla rosenbergi tadpoles, although both species dwell in tropical streams (Noble, 1931). Dunn (1926) noted the reduction of gill size in Jamaican hylids, which breed in water which collects in bromeliads. Likewise, tadpoles of the genus Hoplophryne, which live in water that collects in banana leaves, also have reduced gills (Noble, 1929). Terrestrial embryos of the genus Eleutherodactylus may or may not have external gills, their highly vascular tails serving as important respiratory organs (Lynn, 1961). Among the more unusual are the gills of some Gastrotheca which are large and bell-shaped (Noble, 1931).

Studies of Babak (1907[a], 1907[b]) upon Rana temporaria and Rana esculenta, of Drastich (1925) upon Salamandra maculosa, of Bond (1960) on Salamandra maculosa, Ambystoma opacum and Ambystoma jeffersonianum, of Conant (1958) concerning adult Necturus maculosus and Freeman (1963) on adult Pseudobranchus striatus, all showed an inverse relationship between external gill size and oxygen concentration. Most indicated that external gills increased in size when animals were put into water of lower oxygen concentration. In addition, Conant, Bond, and Freeman all noted that gill movements and expansion, as well as blood supply were correlated with oxygen tension.

Pulmonary respiration is important to the majority of adult amphibians and is found in a number of larvae as well. The lungs of stream dwelling species are reduced in size (Noble, 1931). Savage (1961) has described the well developed lungs of Rana temporaria larvae and still larger ones from a Papuan tadpole; both of these species were taken from standing pools or puddles of water. In the Papuan species, it appeared that tail muscles pressed the lung against the notochord to bring about ventilation.

Savage stated further that:

> The respiratory systems in tadpoles are connected with the ecology in the following way. If a tadpole lives in an environment rich in food, as many temporary or polluted ponds are, it does not need to pump water to get its good, and so does not need large gill filters. To use the oxygen under these conditions, however, it must have large gills, and needs lungs to tide it over emergencies. If, however, it lives in the oligotrophic type of pond, with plentiful and almost constant supplies of oxygen but with a low concentration of food, it needs to pump much water, and so must have large gill filters. With these, gills might not be necessary, because of the large surface of the filters (or rather, in

> view of Strawinski's work, more probably, the associated large operculum). In ordinary ponds, intermediate as habitats, the arrangements might be expected to be intermediate also.
>
> There is a great deal of conjecture in all this, but the microhylids seem to provide examples. Some have such large gill filaments that they trail in the opercular cavity in a way quite unlike those of Rana, others have no gills but have enormous gill filters, for example Glyphoglossus molossus. Some, such as Hypopachus aquae, are intermediate and live in ordinary ponds. Rana temporaria also have a moderate development of gills, filters and lungs, and lives in ordinary ponds.(p. 56)

Among amphibians, we may turn to the work of Strawinski (1956) for an estimation of the relative importance of tadpole respiratory surfaces. In studying the density of capillary networks of Rana esculenta, he reported that most gaseous exchange of early stages took place through the skin, with the internal surface of the operculum also important. It was his belief that the external and internal gills, and vascularized filtering apparatus were of little importance. As the lungs developed, their portion of the total respiratory capillaries increased rapidly, reaching 65 per cent at metamorphosis, the same proportion as in the adult. Vascularization of the skin also increased until metamorphosis, when these capillaries accounted for 34 per cent of the total.

Blood transport of oxygen

Among vertebrates, blood contained within a closed circulatory system is largely responsible for the transport of gases in the body. Hemoglobin pigments, located in red blood cells and responsible for their color, are the main carriers of oxygen in vertebrates. Exceptions

to this are the Antarctic ice fishes, Chaenichthyidae (Ruud, 1959). Blood of the several species of this family is nearly colorless and contains only white cells (1 per cent of blood volume). The cold water in which these fish live has a high oxygen content and the low temperature tends to lower the respiratory rates, so that oxygen diffusion through the naked skin sufficiently supplements that dissolved in the blood to maintain their low rate of metabolism. Small eel larvae also lack hemoglobin until they reach the elver stage (Andrew, 1965) and occasional specimens of the frog, Xenopus laevis, have been found without hemoglobin (de Graaf, 1957; Ewer, 1959).

Among the indicators of oxygen-carrying capacity of blood are measurements of red cell size, erythrocyte counts and determinations of hemoglobin content. Amphibian erythrocyte sizes and counts have been listed by Prosser et al. (1950), Vernberg (1955), Freeman (1963), and Hartman and Lessler (1964). Of all vertebrates, salamanders have the largest red cells, ranging from 30-62 microns in longest dimension. According to these sources, erythrocytes of frogs, which are also oval in shape, range from 18-23 microns in length. The number of red blood cells usually varies inversely to cell size. Counts of salamander cells vary from 28,000 to 197,000 per microliter; those of anurans, from 380,000 to 670,000 per microliter. While one would expect larger cells and higher cell counts to represent greater oxygen-carrying capacity, a trurer measure lies in the direct determination of hemoglobin. Kirkberger (1953), Stuart (1951), and Goin and Jackson (1965) have published values

for hemoglobin concentrations of amphibian blood. These range from 8.7 to 11.5 grams of hemoglobin per 100 milliliters of blood.

In regard to shape, it should be noted that the oval disc form of amphibian erythrocytes presents a greater surface area per unit of volume than would a sphere of like volume, thus presenting a greater surface for gaseous diffusion (Andrew, 1965). Greatest efficiency of oxygen transport among amphibians appears in the genus Batrachoseps, which may have up to 90 per cent erythroplastids or enucleate erythrocytes (Emmel, 1924). Lack of a nucleus makes the cells flatter and thus increases cell count per unit volume, as well as reducing the oxygen uptake of the red cell by the amount consumed by the nucleus (Foxon, 1964).

Transfer of oxygen to respiring tissue

Once oxyhemoglobin is carried to the tissues from the respiratory organs, oxygen dissociates from it and diffuses into the cells. Krogh (1941) has shown that the rate of oxygen diffusion in tissues is variable, but less than half that of water. Less is known of conditions which promote dissociation of oxyhemoglobin of the type found in tadpoles. Since the great affinity which tadpole hemoglobin has for oxygen is not modified by changing pH, dissociation would appear more difficult than it is for oxyhemoglobin of adult erythrocytes (Manwell, 1966).

Oxygen utilization and metabolic rate

Boell (1948) has reviewed earlier work which related to oxygen consumption of early amphibian embryos. Much of this was concerned with respiration of various regions of the gastrula stage. The work of

Brachet (1935) upon eggs of Rana fusca (= Rana temporaria) showed no significant difference between respiratory rates of fertilized and unfertilized eggs.

Among the first studies of oxygen uptake in anuran larvae were those of Helff (1926) and Etkin (1934), working upon Rana pipiens and Rana catesbeiana respectively. Atlas (1938) studied Rana pipiens and Rana sylvatica from fertilization to tadpole states and found that respiratory rates increase from fertilization to overgrowth of the operculum. This was in agreement with the studies of Godlewski (1900) and Bialaszewicz and Bledowski (1915) on Rana temporaria. Parnas and Krasinska (1921) found the rate of oxygen consumption rose sharply at the onset of gastrulation, neurulation, and external gill formation, with no noticeable changes between. Atlas (1938) suggested that the latter conclusions were based upon insufficient data which were adversely affected by temperature fluctuation. It is interesting to note that the respiratory rates obtained by Bialaszewicz and Bledowski for Rana temporaria are similar to Atlas' data for the closely related Rana sylvatica, but somewhat different for Rana pipiens or Rana catesbeiana. Atlas concluded that the increased rate of respiration per embryo was a function of cell number rather than of intensity of respiration of individual cells. The basis for this statement is obscure, since no cell counts were reported, nor was the subject discussed in the main text of the paper.

Relation of Respiration to External Environment

Although a number of studies have dealt with respiration in regard to external factors such as temperature and partial pressure of oxygen, little has been done to relate respiratory form and function of a tadpole to its specific environment. The most thorough study along these lines was done by Savage (1961), who compared several species of tadpoles in relation to feeding and respiration in different environments. His conclusions were quoted previously.

Laessle (1961) noted that the tadpoles of the common Jamaican tree frog, Hyla brunnea, lived in stagnant water of very low oxygen content, which collects in the central reservoirs and leaf bases of bromeliads. He suggested that the agitation of the long tail increased oxygen diffusion into the water and that the large surface area of the tail provided additional respiratory surface. The tadpoles of the other three species of Jamaican tree frogs also develop in the oxygen-poor water which accumulates in bromeliads (Dunn, 1926). Tree frogs, closely related to the Jamaican species, on the island of Hispaniola, are known to breed in streams and torrents (Noble, 1927); the Cuban tree frog, Hyla septentrionalis, is known to develop in brackish water, as well as in cisterns and pools (Grant, 1940).

It may be noted here that Powers et al. (1932), working upon fish respiration in relation to environment, found that "The number of red blood corpuscles is increased with a decrease in the oxygen and by an increase in the carbon dioxide tension of the water and vice versa."

Structure and Function of Frog Liver

Studies of liver structure in frogs have been few; most were considered by Elias and Bengelsdorf (1952). These authors found that the walls separating neighboring sinusoids are predominantly two cells thick in frogs, but only one cell thick in mammals. Similarly, relatively little experimental work has been done on the excretion of bile pigment by frog liver. Nisimaru (1931) carried out perfusion experiments on the liver of Rana catesbeiana. He found that the rate of bile pigment excretion changed when blood pressure in the liver circulation was altered.

Papers concerning frog liver structure or function do not aid in the explanation of chlorosis in these forms.

SOURCES AND CARE OF TADPOLES AND FROGS

The live frogs and tadpoles used for most of this study were collected from their normal habitats during field trips to the West Indies and South America. The species included: Hyla septentrionalis collected from Grand Cayman and Southern Florida; Hyla brunnea, Hyla lichenata, Hyla marianae, and Hyla wilderi from Jamaica; Hyla dominicensis, Hyla vasta and Hyla heilprini from Haiti; Leptodactylus albilabrus of Puerto Rico; and Hyla maxima and Pseudis paradoxus from Surinam. Incidental observations were made of Phyllomedusa tadpoles on Trinidad and several other hylid species in Surinam. Collections were made at the following places during the months indicated: Jamaica -- June-July, 1965; August, 1965; October, 1965; May, 1966 and August, 1966; Haiti -- October, 1965; May, 1966 and August, 1966; Grand Cayman -- July, 1965 and October, 1965; Puerto Rico -- May, 1966; Southern Florida -- July, 1966; Trinidad -- July, 1966; Surinam -- July, 1966.

Tadpoles of the species listed above were collected with a tea strainer or small dip net. They were transferred to plastic bags which were partially filled with water from the immediate environment of the tadpole. When suitable tap water was available, it was used to replace water brought from the field. Thus the frog larvae were transferred to water which was apparently free of noxious substances, detritus, and other organisms. Water was poured off and replaced with fresh tap water as often as required, usually at one- to three-day intervals. Tadpoles did not show marked reactions to addition of tap

water from localities in South America, the West Indies, or Southern Florida. Care was taken to keep the temperature of the fresh tap water about the same as that which had been removed.

Upon returning to the laboratories in Gainesville, Florida, the larvae were transferred to culture dishes of appropriate size. Since Gainesville tap water was found to be lethal to both Hyla brunnea and Hyla septentrionalis, spring water or Holtfreter's solution was ordinarily substituted. During May, 1966, Holtfreter's solution proved unsatisfactory and its use was discontinued. At one point, the spring water appeared harmful and filtered pond water was used in its place.

By avoiding direct sunlight wherever possible and using other precautions, tadpoles were maintained in the field with few losses due to excessive temperature. At the University of Florida, the culture dishes containing the tadpoles were moved from one laboratory to another in an attempt to maintain a moderate temperature; however, groups of tadpoles were exposed to water temperatures between known extremes of 16° C. and 28° C.

Thus, with reasonable care, most of the tadpoles were collected and transferred to Gainesville with little difficulty. A notable exception was the Hyla heilprini tadpoles, which did not survive more than three days after capture. Of the other species, most demonstrated an ability to live for two weeks or more without being fed. However, in the laboratory and whenever possible in the field, the tadpoles were fed Gerber's strained foods at regular intervals. Hyla septentrionalis, Hyla dominicensis, Hyla vasta, and Leptodactylus albilabrus were fed a

mixture of peas, carrots, and spinach. Hyla brunnea, Hyla lichenata, and Hyla marianae were fed strained egg yolk. Tadpoles were maintained satisfactorily for two months or more on these diets. Of those on the above diets, individuals of all species but Hyla septentrionalis reached metamorphosis under laboratory conditions. Following metamorphosis, the young frogs were kept in covered jars, and were fed termites and other insects.

Adult frogs were collected by hand, using a flashlight at night to locate them at their calling locations, or by searching for them in their resting sites during daylight hours. They were transported in jars or in plastic or cloth bags. In the laboratory, they were kept in jars or terraria. Crickets or other insects were used to feed the adult frogs at approximately weekly intervals.

In addition to live specimens, hundreds of preserved frogs and tadpoles were surveyed for unusual characteristics. Most of these specimens were of West Indian hylids and were obtained from the museums mentioned under Acknowledgments.

I. CAUSATIVE PIGMENT AND INCIDENCE OF CHLOROSIS

Pigment Identification

During the present study, filtered tissue extracts and body fluids were tested spectrophotometrically and chemically. Figures 2-6 show that the spectral characteristics of several of these green fluids are very similar to those of biliverdin (Figure 1). Addition of fuming nitric acid to the fluids was followed by the succession of colors known as the Gmelin reaction, generally considered to indicate the presence of bile pigments. These two tests, along with characteristics of color and solubility, appear to eliminate from consideration all known green pigments except the two naturally occurring green bile pigments, biliverdin and mesobiliverdin. Since green bone is rapidly bleached by concentrated sulfuric acid, mesobiliverdin does not appear to be present, since it is stable in this substance. Thus, it was independently concluded during the present study that biliverdin is the pigment primarily responsible for the green color of these frog tissues.

Survey of green pigmentation among Neotropical anurans

During the course of several field trips to the West Indies and one to Surinam, it was possible to study a variety of tropical anurans under natural conditions. Many of these did not exhibit any green color, while others showed different levels of green pigmentation in the tissues and body fluids. Observations made during the present

study and by others are summarized in Tables 1 and 2. Points to be noted from Table 1 include the fact that the color of the bile was usually green. In many individuals, the bile was so concentrated as to appear blue when seen through the gall bladder wall. When soft tissues were green, it was usually a rather general phenomenon and often was accompanied by a relatively high concentration of plasma pigment, a condition which might be termed chlorosis.

Sex and its relationship to chlorosis in frogs

Due to the relatively small number of individuals collected from each species, it is difficult to make a generalization concerning the relationship between chlorosis and sex. Chlorotic and non-chlorotic individuals of both sexes were among specimens of Hyla septentrionalis from Grand Cayman. Table 2 shows several species in which all tadpoles observed had green pigment at the time of metamorphosis or before. The general occurrence of biliverdin in these species indicates that the sex of the individual is not important to the development of chlorosis at this stage. The presence of green pigmentation in calling males of several species and in eggs of certain Hyperolius, Agalychnis, Centrolenella, Pseudis and Hyla indicates that sex is not a very important factor in the development of chlorosis.

Age distribution of chlorotic anurans

Among tadpoles, no green pigmentation was noted in young tadpoles of Hyla brunnea, Hyla boesemani, Hyla dominicensis, Hyla leucophyllata, Hyla maxima, Hyla rubra, Hyla septentrionalis, Hyla vasta, or Hyla wilderi.

When green pigmentation was present in tadpoles, usually it made its appearance at the onset of metamorphosis and reached a peak upon the completion of metamorphosis. All of the species of Hyla listed in Table 2 are from the West Indies and they present similar appearances at the completion of metamorphosis, except for Hyla marianae. Most of their skeletons were green, particularly the limb bones and vertebrae; the green pigmentation was not so pronounced where the marrow maintained its hemopoietic function; melanin pigmentation had developed, especially on the dorsal side; pigmentation of the ventral side was not well developed in Hyla brunnea, Hyla lichenata, or Hyla marianae, all of Jamaica (stomach contents could be seen through the skin); a white substance, presumably guanine, had developed in the skin of the other species, which are usually found in more exposed habitats than the Jamaican species; green pigmentation of soft parts was particularly intense in the region of the throat and pectoral girdle; the small size of most of these species of tadpoles made it difficult to determine the color of the plasma. In Surinam, the tadpoles of Pseudis paradoxus were found to be darkly pigmented, externally by melanin and internally by biliverdin and other pigments. The plasma of this species is green prior to metamorphosis. The coiled intestines of these gigantic tadpoles are packed with green plant materials and fill most of the body cavity.

During the present study, a young Rana heckscheri collected near Gainesville, Florida, at the completion of metamorphosis was found to have gray-green bone marrow. This appeared to be a stage which represented degenerating red marrow of the tadpole. This color appeared

to be restricted to the marrow, since neither the bone nor the plasma demonstrated green pigment. Although the origin of this pigment probably was the same as that of the Neotropical forms, the color was almost obscured by the bone.

Green pigmentation of the adults is summarized in Table 1. It is worthwhile to compare the amount of green pigment in adults with that of tadpoles of the same species, where possible. Tadpoles of Pseudis paradoxus, Hyla dominicensis, Hyla lichenata, Hyla septentrionalis and Hyla vasta generally had much more extensive chlorosis of soft tissues than did the adults of these species. Two adult specimens of Hyla heilprini showed chlorosis as extensive as that of metamorphosing tadpoles, but melanin pigments of the dorsum were better developed in the adult than in the tadpoles. While scores of tadpoles of Hyla brunnea invariably had green pigmentation, dozens of adults showed no biliverdin in tissues, including bone. Green pigment is absent from the tissues of Hyla marianae, which are orange in color.

As indicated previously, there was individual variation of green pigmentation within populations of Hyla septentrionalis. Small species such as Hyla wilderi have bones of a more intense green color than do larger species such as Hyla maxima or Pseudis paradoxus. Calcification of bones tends to obscure the green pigment, as in the larger species, but the color of biliverdin is quite apparent in poorly calcified bones as in the distal limb structure of Hyla wilderi.

Seasonal changes in chlorosis

It should be noted that most of the specimens for the present study were collected from June through August. This period constitutes the main breeding period for most of the species studied, a fact which should be kept in mind when considering the physiology of these organisms.

It is interesting to note that Hyla geographica had green bones, but no green pigment in the plasma. On the other hand, Hyla misera had light green plasma, but white bones. These two examples indicate that high concentrations of green pigment in the plasma are temporary in some species. Different shades of green in plasma and bones of other species tend to support this idea. Concentric layers of different shades were seen in Barrio's (1965[a]) photograph of bone from Lysapsus mantidactylus, and in a femur of Osteocephalus taurinus. Like growth rings of a tree, these suggest a seasonal change in conditions during development of the tissue.

Phylogenetic distribution of chlorotic frogs

An understanding of the phylogenetic relationships of chlorotic frogs to those which lack tissue biliverdin should be of value. However, Tables 1 and 2 indicate no clear boundaries along phylogenetic lines. As previously mentioned, adult individuals of Hyla septentrionalis and Hyla dominicensis may fall into either category. In Hyla brunnea, the pigment is always present in young frogs, but has never been observed in the adults. Hyla pulchella has some populations which are chlorotic and others which are not. Similarly, it can be seen that neither the genus Hyla nor the family Hylidae shows uniformity in this characteristic.

The degree of pigmentation is no more helpful than its presence or absence. Of the species collected in Surinam, Hyla punctata, Hyla crepitans, Sphaenorhynchus aurantiacus and Phrynohyas venulosa had the highest concentration of green pigment of the frogs collected, but they certainly do not constitute a homogeneous group of species. Of the eight species of Hyla that were studied in life in the West Indies, seven had green pigmentation at metamorphosis or later, but Hyla marianae lacks green pigmentation in these stages, being orange instead.

There is at least one relationship between chlorosis and frog phylogeny. Thus far, all chlorotic frogs have been members of only four families. Most of the green species are members of the family Hylidae, while the others are included in the Centrolenidae, Pseudidae, and Rhacophoridae. The chlorotic hylids, centrolenids and rhacophorids are all tree frogs, while pseudids are aquatic for extensive periods. All four of these families have in common an intercalary cartilage between the ultimate and penultimate phalanges of the digits.

A close relationship between the four families is unlikely even though they have the intercalary cartilage, green pigmentation and tropical distribution in common. Hyperolius, the African genus of rhacophorid frogs which has green eggs, has a firmisternal pectoral girdle and diplasiocoelous vertebrae which distinguishes it from chlorotic frogs of the other three families, which are procoelous, have an arciferal pectoral girdle and are Neotropical. There appears to be considerable agreement that the presence of intercalary cartilages in

these families is the result of parallel evolution. All things considered, there appears to be no clear relationship between phylogeny and chlorosis among frogs.

Geographic distribution

Frogs with green tissues or eggs appear to be tropical or sub-tropical in their distribution. Below is a list of green pigmented frogs according to the countries or regions in which they are found. Sources of information are given in Table 1.

Central Africa: Hyperolius sp.

Jamaica: Hyla brunnea, Hyla lichenata, Hyla wilderi.

Hispaniola: Hyla dominicensis, Hyla heilprini, Hyla vasta.

Cayman Islands (presumably also Cuba and Bahama Islands): Hyla septentrionalis.

Mexico and Central America: Agalychnis dacnicolor, Centrolenella albomaculata, Centrolenella granulosa, Centrolenella ilex, Centrolenella spinosa, Centrolenella prosoblepon, Centrolenella pulveratum.

Northern South America, including Brazil: Lysapsus limellum laevis, Pseudis paradoxus, Anotheca coronata, Hyla albofrenata, Hyla albomarginata, Hyla boesemani, Hyla calcarata, Hyla crepitans, Hyla cuspidata, Hyla geographica, Hyla langsdorffi, Hyla maxima, Hyla misera, Hyla punctata, Osteocephalus taurinus, Phrynohyas venulosa, Sphaenorhynchus, Trachycephalus nigromaculata, Centrolenella vanzolinii.

Argentina: Lysapsus limellum limellum, Lysapsus mantidactylus, Pseudis paradoxus platensis, Hyla berthae, Hyla nasica, Hyla pulchella,

Hyla phrynoderma, Hyla punctata rubrolineata, Hyla raniceps, Hyla siemersi, Hyla squalirostris, Hyla trachytorax, Phrynohyas venulosa.

Judging from this list, it appears that nearly all green pigmented frogs are to be found in the Neotropical Zone. It should be noted that, except for the works of Barrio (1965[a], 1965[b]) and the present writer, the references to green tissues or eggs of frogs are few, and these are often obscure. Although a number of individuals have volunteered personal observations concerning coloration, there appears to be a general reluctance to publish such observations. It seems quite possible, if not likely, that additional records will be forthcoming from Africa or other parts of the Old World tropics.

II. CONSIDERATION OF ECOLOGICAL FACTORS ASSOCIATED WITH CHLOROSIS

In attempting to determine the cause of a condition such as chlorosis, ecological factors should be considered. It has already been noted that the majority of chlorotic anurans are tree frogs which are restricted to tropical or sub-tropical regions. However, not all tropical tree frogs have green tissues; conversely, the chlorotic pseudids are not tree frogs, but are aquatic. Since the tropical climate and general habitat (aquatic, terrestrial or arboreal) do not completely account for either presence or absence of chlorosis, one should consider the importance of specific habitats, as well as behavioral adaptations to such environments. This section includes a summary of information concerning specific habitats and the frogs associated with them. Greater emphasis was placed upon ecological study of breeding sites because of their accessibility and the appearance of chlorosis during the larval stages. Special attention was given to those factors which might be related to hemoglobin or red cell formation and function, including oxygen tension, temperature and iron concentration. Additional factors were surveyed in order to find conditions which were markedly different from those ordinarily encountered by anurans.

Methods of Study

Concentration of dissolved oxygen in the habitats of tadpoles was measured with a Precision Scientific Portable Oxygen Analyzer, which was calibrated in air.

Temperature readings of tadpole habitats were taken with the thermistor component of the oxygen analyzer wherever possible; otherwise, they were made with standard mercury thermometers, graduated from -10° C. to 100° C.

Ferrous and total iron, and phosphate concentrations of water were measured by colorimetric methods using a Hach portable colorimeter and the appropriate techniques (Hach Chemical Company, no date). Salinity, alkalinity and hardness were measured by titrametric methods, using the appropriate kits and techniques developed by the La Motte Chemical Company. Water pH was measured to the nearest half unit by means of pHydrion paper.

Observations of Frog Habitats and Behavior

During this study, frogs were collected in a variety of habitats from tropical rain forest to open, grassy areas to residential districts. Although eggs and tadpoles were found in or near the environments of the adults of their respective species, the larval environs were more uniform in appearance. Most of the tadpoles were found in standing water, but several species of tadpoles were found in flowing streams. While the temperature and dissolved substances of these habitats were found to vary considerably, one may satisfactorily divide tadpole habitats into flowing stream and standing water types. Within the latter type, special consideration will be given to the bromeliad microhabitat of Jamaican tree frogs. Ecological data collected during this study are presented in Tables 3-5.

The bromeliad microhabitat

Members of the pineapple family, Bromeliaceae, ordinarily do not constitute the dominant plants of a habitat, although they may be an important part of the flora. On the West Indian island of Jamaica, the bromeliads have undergone considerable adaptive radiation (Dr. Richard Proctor, personal communication). They may be large or small, epiphytic or terrestrial, and are found in shaded and open areas. This appears to be a very fortunate circumstance since the water which is caught in the leaf bases and central reservoirs of bromeliads is the most reliable supply for small animals, including the four species of tree frogs on Jamaica.

The importance of the close relationship between the Jamaican tree frogs and the bromeliads should not be underestimated. Perkins (1948) noted that the water level in the bromeliads ("wild pines") is maintained by dew which condenses and runs down into the reservoir in the center of the plant; very little direct sunlight tends to reduce evaporation from the wild pines. She states further (p. 87), "In view of the many creatures that depend on the wild-pine for moisture it would seem that these plants hold an important place in the economy of the countryside, for surely our wildlife would be largely depleted during a severe drought, were it not for these hidden stores of water." My observations during the drought which continued into July, 1965, substantiate this position. Except for an occasional pool in stream beds, or the largest rivers which continued to flow, there was no surface

water other than that in the bromeliads. Steepness of the hills and porosity of the limestone substrate are partly responsible for rapid run-off of water.

Observations of the adults of the three smaller Jamaican hylid species indicate that they prefer to be covered by water, at least in a lighted area. Under these circumstances, they may remain completely immersed for minutes at a time, and then slowly rise until only the external nares and eyes protrude above the surface. It is interesting to note that the four Jamaican species differ from their relatives of Cuba and Hispaniola in having a more truncate snout with the external nares at the most anterodorsal point (Dunn, 1926). This may be interpreted as an adaptation to living in the reservoirs of bromeliads.

Laessle (1961) studied the ecology of Jamaican bromeliads, in which he found the following ranges for the water which they contained: dissolved oxygen, 0.0 - 8.0 ppm; dissolved carbon dioxide, 4.0 - 67.0 ppm; pH, 4.0 - 7.0; temperature, 17.5° - 30.0°. He estimated the maximal quantity of water in the reservoir of a large bromeliad at 200 milliliters.

Hyla brunnea. -- The brown tree frog of Jamaica is the most widely distributed species of Hyla on the island. It is absent from the Blue Mountains above 1600 meters elevation, and from arid areas such as Kingston and the Hellshire Hills along the south coast (Lynn, 1940). This frog is most likely to be found in localities where the large tank bromeliads, including species of Hohenbergia and Aechmea, are readily available as breeding sites and resting places. While this frog

may be found in bromeliads which grow at ground level on shaded, limestone hillsides or in the epiphytes high above ground in well developed forests, they are frequently found in towns or among the trees which line the roads in agricultural areas. Like the other tree frogs of Jamaica, Hyla brunnea appears to be largely dependent upon the bromeliads for the water which is retained at the leaf bases, but several Jamaicans have told me that the brown tree frog is found among banana leaves and stalks.

Breeding habits of Hyla brunnea are unusual in some respects, particularly as they relate to adaptations to life in the bromeliads. The breeding season begins during May, as indicated by a record cited by Dunn (1926). Panton (1952) has stated that the strongest choruses of the tree-toad are heard at the end of May or beginning of June, but that the time fluctuates because of weather conditions. During the latter part of May, 1966, and early June, 1965, I noted very few tadpoles, but found eggs more common than later in the year. Tadpoles were not uncommon during October, 1965. The eggs of Hyla brunnea are deposited in the central reservoir of bromeliads in most cases, but eggs or tadpoles may be found at the bases of outer leaves; on one occasion, eggs were found in water that had collected on a hollow branch.

Development of the tadpole stages has been described by Schreckenberg (1956), who studied the embryonic development of the thyroid gland in Hyla brunnea. The mode of development differs little from that seen in Rana and hylids of the United States. The main differences appear to be the result of adaptations to the bromeliad habitat.

The jelly mass from which the tadpoles hatch remains in the bromeliad reservoir longer than do the tadpoles themselves. Panton (1952) has suggested that the presence of the jelly reduces evaporation and moderates temperature changes. The low pH of the water probably prevents the rapid decay of the jelly mass or infertile eggs. In his micro-limnological study of Jamaican bromeliads, Laessle (1961) found the following ranges of readings in five bromeliads which contained eggs or tadpoles of Hyla brunnea: dissolved oxygen, 0.03 - 2.3 parts per million; dissolved carbon dioxide, 23.0 - 41.0 ppm; pH, 4.0 - 4.5; temperature, 23.0 - 25.0° C. Additional measurements made during the present study increased these ranges to: dissolved oxygen, 0.03 - 2.7 ppm; pH, 4.0 - 6.5; temperature, 23.0 - 28.0° C.

During the present study, the contents of a number of bromeliad reservoirs were poured into waterproof containers. When jelly masses without eggs were present, they were not firm and had a tendency to separate into capsules 1-2 centimeters in diameter. These probably represent capsules which contained four to six eggs each, as mentioned by Dunn (1926). Within the bromeliad, the mixture of water and jelly has the consistency of glycerine, as mentioned by previous writers. While I have noted tadpoles, especially small ones, moving about near the surface on several occasions, I have also noted larger ones moving vertically within the reservoir. The larger individuals tend to remain under leaf fragments when the reservoir is exposed to the sun. They come to the surface at intervals to take air and then return to lower depths. This respiratory behavior apparently was not observed previously, since

Dunn (1926) was unable to reconcile the reduced gill structure with the low oxygen content of the environment. Lungs are seen on either side of the vertebral column, and appear like small bubbles. Lungs are present in very small tadpoles as well as older ones. They appear to be used as accessory respiratory structures when branchial and cutaneous respiration are insufficient. In this respect, the tadpoles would be similar to the Australian lungfish, which utilizes pulmonary respiration largely at night when it becomes more active (Grigg, 1965). While the long, narrow tails of Hyla brunnea tadpoles probably are important to cutaneous respiration as Laessle (1961) indicated, it seems likely that their primary purpose is to propel the organisms through their viscous environment.

The diet of Hyla brunnea tadpoles consists largely of frog eggs, as noted by Dunn (1926) and Laessle (1961). Most often the eggs are probably those of its own species, since it is by far the most common tree frog of Jamaica. In addition, it is the only hylid species known from the eastern third of the island so that any hylid eggs found in Hyla brunnea tadpoles there, would have to be of the same species (Laessle, 1961).

Several structural characters of the tadpoles can be correlated with their unusual diet. The digestive tract is expanded into a sac in which eggs may be found more than a week after the last feeding. It is relatively straight, not coiled as in vegetarian tadpoles. Since the gills are not important in collection of food, their structure is

simplified. Finally, the mouth has only a single row of teeth about it (Dunn, 1926), since these are not needed for scraping food from surfaces.

The length of time required for development from fertilization to metamorphosis is not known since neither Lynn (1940) nor I was able to follow a single group through the entire period. From Lynn's work, nearly a week passes between fertilization and hatching. After hatching, at least a month probably passes before metamorphosis is completed. While tadpoles have been kept in captivity for more than six weeks, it is likely that metamorphosis could be completed approximately six weeks after fertilization under optimal conditions.

At the time that the forelegs penetrate the opercular fold, the green pigmentation was always quite obvious. Jarring of the bromeliad or container in which such tadpoles were present caused them to climb upward very rapidly. They have no difficulty in climbing out of a bromeliad reservoir, a water glass, or a plastic bag. Once they leave the water, the tail is resorbed in twenty-four to thirty-six hours. This is in agreement with the finding of Schreckenberg (1956) that there is intense thyroid secretory activity and sudden release of colloid from the thyroid gland at the stage of tail resorption. The dark green pigmentation of soft tissues in the gular region remains for two weeks or more after metamorphosis and then gradually fades away in the living animal. Green pigmentation of bones remained as long as the young frogs lived, or about six weeks after metamorphosis, in the laboratory. In nature, no immature

frogs were found in bromeliads, ant or termite nests, or elsewhere, so that the post-metamorphic development and ecology of the young are unknown.

Hyla lichenata. -- This giant tree frog, which attains a length of 117 millimeters, is restricted to the central and western hills of Jamaica, generally above 300 meters in elevation. Since this frog is rarely seen and less often collected, knowledge of its habits and distribution is based largely upon hearing its distinctive snoring call (see Lynn, 1940).

In regard to the habits of this species, Panton (1952) first found one in a bromeliad on a dead candlewood tree in woodland, but all subsequent specimens were taken from hollow trees. Dunn (1926) collected three of these frogs from small hollow trees with openings 4 to 12 feet above the ground, but noted that they ordinarily call from greater heights. He stated further that the "bony head is obviously of use in plugging the hole after the frog is inside." Lynn and Dent (1943) traced the unmistakable call of this species to a clump of bamboos near Chapelton. A female specimen of Hyla lichenata was collected by Dr. Thomas Farr of the Institute of Jamaica, near the entrace to St. Clair Cave, St. Catherine Parish on June 19, 1965. During several days in captivity at the Institute of Jamaica, this individual deposited a number of eggs. It showed no interest in a cockroach which was offered as food. This individual produced a skin secretion which became gum-like on the hands of those who held it. Gosse (1851) recorded an instance where the skin secretion of this species caused severe irritation to the human eye.

During my visits to Jamaica I heard Hyla lichenata as far east as localities near Moneague, Ewarton and Lluidas Vale. These calls could be heard for nearly half a mile and invariably came from wooded slopes. This species did not venture out into flat, open valleys, as did Hyla brunnea. In attempting to collect this species while it called at night, I found the vocal individuals to be in trees, with one exception. North of Mandeville, it seemed that one frog was calling from underground. This observation, along with the presence of Dr. Farr's specimen near a cave entrance, leads one to suspect that these large frogs may take refuge in cave entrances and crevices in limestone, both of which are present in quantity in the range of Hyla lichenata.

Dunn (1926) found four tadpoles of Hyla lichenata in a bromeliad 20 feet up in a small tree, in August, 1925. I collected two large tadpoles of this species from a large bromeliad situated on the ground under the shade of small trees. These were taken in Rose Valley on October 25, 1965. These large specimens could be distinguished from tadpoles of Hyla brunnea by their large size, relatively shorter and more muscular tail, and slightly different mouth structure. They were only slightly darker than Hyla brunnea tadpoles, not black as were those collected by Dunn. Like other Jamaican hylid tadpoles, they feed upon frog eggs. One of my two specimens underwent metamorphosis and died about six days later. At the time of its death, it weighed 660 milligrams and had a snout-vent length of 18.7 millimeters. This individual developed green pigmentation, similar to that of Hyla brunnea, in its bones and other tissues.

Hyla wilderi. -- This small, green species is said to be quite common in bromeliads about Mandeville, Jamaica, "and appears to have a fairly wide range in the central part of the island above 1,000 feet" (Lynn, 1940). Dr. Albert Laessle (personal communication) found this species common on Juan de Bolas mountain, from which there is a large series of specimens in the collection of the Museum of the Institute of Jamaica. I was unable to collect a specimen there although I heard a call which may have been of this species. The call of this species is a faint clicking sound, which becomes louder as the frog continues to call. However, in localities near Moneague and Fishbrook, I traced similar calls to frogs which appeared to be of the genus Eleutherodactylus. Unfortunately, both of these frogs escaped. During August, 1965, I collected three adults from the Cockpit Country four miles north of Quickstep, and a tadpole near Moneague. Another individual was taken from Crown Lands in the Cockpit Country during May, 1966.

During the present study, adults of this species were found in large as well as small bromeliads. My experience was similar to Dunn's (1926) in that this species appeared most often in open woods, usually with a southerly exposure. While Dunn (1926) collected one Hyla wilderi tadpole for each seven of Hyla brunnea, only one Hyla wilderi was collected during the present study, along with more than 500 Hyla brunnea tadpoles. The tadpole was collected from a large bromeliad in one of several trees in a pasture. It was not darker than the Hyla brunnea tadpoles collected at the same time, but did appear to have a greater density of melanophores. The hind limbs on this specimen did not have

green pigment. Tadpoles of Hyla wilderi have been collected in March, April and August (Lynn, 1940).

The tadpole of Hyla wilderi has poorly developed gills, similar to those of Hyla brunnea, and like other Jamaican tadpoles, its body is depressed, and has no fin.

Dunn (1926) found that the specimens which Barbour (1910) considered pale green young of Hyla brunnea were actually Hyla wilderi. In fact, Dunn believed that the non-ossified head and green bones of adult Hyla wilderi represented a neotenic condition because of its resemblance to the young of Hyla brunnea and related forms. He suggested that Hyla wilderi, as well as Hyla lichenata and Hyla marianae arose from a frog of the Hyla brunnea type through sympatric speciation, because of differential growth rates.

Hyla marianae. -- Of the Jamaican hylids, least is known of Hyla marianae. Dunn (1926) originally considered these yellowish-green or greenish-brown frogs to be the young of Hyla brunnea, but then recognized them to be of a different species, which he described. The range of this species appears to be the most restricted of the Jamaican hylids. Most of the specimens have been collected in or near the Cockpit Country of west-central Jamaica; two specimens came from Hollymount, Mt. Diabolo, further to the east (Lynn, 1940; Goin and Cooper, 1950). This area has a combination of high elevation (above 400 meters), greater rainfall and less disturbance of habitat than surrounding areas. Limestone cliffs covered by lianas and hillsides covered with jagged, honeycomb limestone usually form the steep sides of the "cockpits." Vegetation on these

hillsides varies from sparse, scrubby growth, to saplings which shade an understory of terrestrial bromeliads, to large trees. The areas of sparse vegetation usually are higher on the hills and have a more southerly exposure than do the areas of denser vegetation. Dunn took his frogs from "wild pines" in rather thick woods. I collected one male, two females and four metamorphosing tadpoles of this species from small, compact bromeliads along the border between a sunny clearing and a thick growth of saplings on the slope above. Hyla wilderi and Hyla brunnea adults were collected from the same site.

Three of the four Hyla marianae tadpoles were in the same small bromeliad, but between different sets of leaves. All of them were engorged with eggs. The tadpole mouthparts had been lost and the mouths of these tadpoles were quite wide. Although the adults of this species (28-38 mm) were slightly more than half the length of Hyla brunnea (56-57 mm) adults, the tadpoles of Hyla marianae appeared to be about the same size as Hyla lichenata tadpoles at metamorphosis. Like the tadpoles of Hyla brunnea, those of Hyla marianae were quite active and tended to move upward at metamorphosis. The color of these tadpoles was brown. The adults which I collected could change from orange to brown and back. The bones of adults and tadpoles were orange with no trace of green in the soft tissues.

Pond habitats

During the course of this study, a relatively large number of frog species were found breeding in bodies of standing water. In addition to the bromeliad habitat already considered, such environments included:

ponds, pools, puddles and wheel ruts filled with water; roadside ditches and drainage canals; flooded, grassy meadows; and cattail marshes. These waters varied widely in size, amount of exposure to sun and wind, and in water characteristics (Tables 4 and 5). Temperatures at mid-day ranged to 40° C. in shallow open waters, but generally were less than 35° C. when exposed to direct sunlight for only a few hours; where direct sunlight did not reach the pools, temperatures remained under 30° C. Oxygen concentrations varied from less than one part per million to ten parts per million and appeared to be affected by air currents and photosynthesis as well as temperature.

The pH of standing surface waters in the West Indies appeared to average somewhat higher than the pH of water in bromeliads in the region. This may be due to the fact that the surface strata of most of the West Indies consist of limestone. At a locality near Juan de Bolas in Jamaica (Table 4, Habitat 7), the pH of water in three bromeliads without an obvious layer of dust on the leaves ranged from 5.5 to 6.5; three bromeliads near the road had a heavy covering of dust on the leaves and the pH of the water in the reservoirs was 7. Presumably the dust from the limestone gravel of the road reduced the acidity of water in bromeliads when it was washed into the central reservoirs by rain or dew. Other water characteristics do not show definite patterns (Tables 4 and 5).

It is difficult to make a distinction between exposed and shaded pool habitats. There may be greater difficulty in assigning a particular species to one or the other. Hyla dominicensis and Hyla

septentrionalis are quite opportunistic in regard to selection of breeding areas. However, in Surinam, several species appeared to be associated almost exclusively with forest habitats. Included in this group are Hyla geographica, Hyla calcarata, Hyla lanciformis, and Osteocephalus taurinus, all of which are medium-sized and brown dorsally, and the large species, Phyllomedusa bicolor, which is green above.

Hyla geographica. -- A silent male of Hyla geographica was collected from vegetation on a rocky ledge of Princess Irene Falls, Brownsberg, Surinam. Wooded slopes were present on both sides of the falls and the stream which flowed over it. Tadpoles were present at the base of the falls, but these were believed to be larvae of Hyla maxima, which was calling at the locality. This specimen of Hyla geographica had light green bones but no other green organs.

Hyla calcarata. -- The single female was collected from a bridge over a small stream which flowed into a swamp. This individual had green bones, but no other green pigmentation.

Hyla lanciformis. -- Most of the specimens of Hyla lanciformis were collected in wooded areas. One was collected while calling near the edge of the forest in the vicinity of temporary pools. No eggs or tadpoles were found. Goin and Layne (1958) stated that its note was regularly heard in open lands such as wet meadows near Leticia, Colombia. They noted that the specimens collected were perched on bushes or other plants one or two feet above ground. Four specimens from Surinam lacked green pigmentation in bone or other tissues.

Osteocephalus taurinus. -- A female with green bones was taken from the trunk of a tree on the upper coastal plain in Surinam. The tree was beside a road through a well-developed forest; a stream and standing puddle were nearby.

Bokermann (1965) has recently given the first description of the breeding habits of Osteocephalus taurinus. Rainfall in the vicinity of Marmelo, in forests of western Brazil, is strongly seasonal, but reaches 2250 millimeters annually. Osteocephalus taurinus choruses were heard at the time of the first rain in November. Two of the breeding pools used by the species were in the forest; in one of these ponds, there was breeding activity at 1:00 P.M. The surfaces of these ponds were covered by eggs of this species which were 5 millimeters in diameter, including the jelly envelopes. A temperature reading from one of them indicated the water temperature to be 27° C., while the air temperature was 34° C.

Of particular interest was a third breeding pond found by Bokermann. This pool was in an open area and contained the dead crown of a tree which made difficult the capture of frogs beneath the branches. However, as the collectors approached the pool, the frogs followed their habit of rapidly climbing upward to escape capture until they reached the ends of the tree's bare branches. After eight minutes in this exposed position, the first frog fell into the pool, motionless but not dead. Although its skin had dried in several places, it recovered. Air temperature in the sun was 46° C. and the water temperature was 32° C. at several points of the pond. Two days later, evaporation had greatly

reduced the size of this pond. Water temperatures rose to 38° C. and the embryos were dead.

Phyllomedusa bicolor. -- This is a large arboreal species which appears to breed near stagnant water which may be in open areas with some exposure to the sun. One adult male was collected from the surface of a main road on the upper coastal plain of Surinam. Another was heard calling from a site high up in a tree in the forest near Powakka. The male which was collected had no green pigmentation in the tissues. As with other members of this genus, this species moves very slowly, never hopping, but walking on all fours. The whitish color of muscular tissue in this individual may be an indication of poor vascularization or a relative lack of myoglobin.

Phyllomedusa eggs and tadpoles believed to be those of Phyllomedusa bicolor were observed at the New York Zoological Society's biological station at Simla, Trinidad. The large, yellow eggs were attached to the upper side of a large shaded leaf which overhung a pool. Water lilies and water hyacinths covered part of the surface of this small, ornamental pool, which harbored the Phyllomedusa tadpoles of various sizes. The tadpoles were slaty-blue in color, much the same color as the adult dorsum in preservative. They fed upon aquatic vegetation and were observed coming to the surface. Lutz (1954) stated that the larvae of Brazilian species of Phyllomedusa use their rudimentary lungs as hydrostatic organs. Movement of these tadpoles is accomplished with a minimum of effort. The main propulsive structure is the narrow, upturned tip of the tail which vibrates constantly. This causes a slow, steady

movement of the larva; occasionally, the tadpole moves more rapidly by using the entire tail to propel itself. Both the tadpole and the adult of this species give the impression of being quite slow. The assumption might be made that this indicates a low rate of metabolism, but this was not measured in frog or larva. As in the adult, no green pigmentation were found in the tadpole.

A relatively large number of frog species was found in exposed habitats where there was standing water. Neither tadpoles nor adult frogs were seen often in such habitats during daylight hours. Tadpoles generally avoided bright sunlight by swimming under aquatic vegetation.

Elachistocleis ovale. -- In Surinam, this small microhylid was found calling from shallow roadside ditches containing standing water. The ditches had a moderate growth of grass and were generally exposed to the sun. This species was found in Habitat 25 (Tables 3 and 4). Neither eggs nor tadpoles were found. The three adults examined did not show any indication of green tissues (Table 1).

Pseudis paradoxus. -- This highly aquatic medium-sized frog and its exceptionally large tadpoles were found in roadside ditches and drainage canals in Surinam. Water in these ditches usually exceeded 50 centimeters in depth, was semi-permanent and stagnant. Vegetation in this habitat was varied and served as food for Pseudis tadpoles. While the eggs of this species were not seen in Surinam, Gans (1956) found that this species lays its eggs in a frothy mass which floats on ponds in Trinidad. The black tadpoles grow to more than 15 centimeters in total length, a third of which is body length. Body length of the largest

tadpoles approximates that of the adults. Mr. Walter Polder (personal communication) observed that Pseudis tadpoles grow to this large size during the course of a single rainy period of four to six months. Thus, the growth rate of this species is phenomenally high. In comparison with other species, the adults of which are the size of adult Pseudis, metamorphosis of this tadpole is greatly delayed. The slow, deliberate movements of this tadpole reflect its low metabolic rate (Table 9). Although typical habitats (such as Habitats 23 and 24 of Tables 3 and 4) of this species were visited during the day, neither tadpoles nor frogs were seen. Both forms appeared to be more active at night, when they could be seen floating nearly vertically in the water. Its dark pigmentation, low metabolic rate, and perhaps decreased diurnal activity are probably important adaptations of this tadpole to its exposed and warm environment. The adult can swim and hop quite rapidly. Thousands of individuals were seen hopping across the road on a rainy night toward the end of the wet season (late July).

Both the tadpoles and the adults of this species have green pigmentation in the tissues, but it is particularly pronounced in the tadpoles. In the larger tadpoles, green pigment permeates the tissues so that they appear almost as dark internally as externally. Development of green pigmentation in this species does not appear to be related to metamorphosis, since it is well developed before that time. Green pigmentation in the adults appears limited to the well-calcified bones. Their other internal tissues are usually light in color and the blood is bright red. Thus, there is a marked difference between adult and tadpole with regard to green pigmentation.

Smaller hylas. -- In Surinam, several species of small or medium-sized hylids were found calling or breeding in open, grassy ponds or flooded fields with a dense growth of weeds. Both of these situations appeared to be temporary due to increased rainfall at that season (July).

Two very small species, Hyla misera and Hyla minuta, as well as Hyla boesemani, Hyla egleri, Hyla leucophyllata, Hyla rubra, Hyla punctata, and the medium-sized Hyla crepitans, were included in this group. Goin and Layne (1958) recorded Hyla rubra, Hyla misera, Hyla punctata, Hyla leucophyllata, and Hyla lanciformis from similar habitats near Leticia in southern Colombia.

While several of these species were found in the same pond in Surinam, they often had different habits. Hyla misera usually called from a perch on stalks of grass which were surrounded by water; Hyla crepitans was found floating in the water near clumps of grass; and Hyla boesemani called from the grasses at the edge of the same pond. Within this group, Hyla rubra was most likely to be found in the vicinity of Paramaribo, becoming less frequent at higher elevations to the south. Hyla punctata was found perched on weeds in a flooded field and Hyla leucophyllata called from bushes along a drainage canal.

Of the eight species collected in Surinam in this habitat, Hyla egleri (one individual), Hyla leucophyllata (three), Hyla minuta (two), and Hyla rubra (four) showed no green pigmentation. Hyla boesemani (one), Hyla crepitans (two), and Hyla punctata (two) had green bones and the latter two species had green plasma as well. The single specimen of Hyla misera had light green plasma.

Phrynohyas venulosa. -- This is a medium-sized species which has a well developed pattern of melanin pigmentation which largely obscures the extensive internal green pigmentation of bones, soft tissues, and plasma. The poisonous secretion from the skin of this species is well known; breaks in the skin make one particularly susceptible to inflammation caused by contact with this secretion (Goin and Layne, 1958). Phrynohyas venulosa was heard or collected from swamps, shallow roadside ditches near forests or thickets, and from residential areas in Surinam. Tadpoles of this species were collected from standing water in roadside ditches near wooded areas.

Zweifel (1964) described the life history of Phrynohyas venulosa from Panama. The eggs were laid on the surface film of marshy ponds. When brought into the laboratory, they hatched in less than a day and metamorphosis took place after 37 days. The tadpole was of the pond type, with globose body, lateral eyes, and well-developed tail fin. The early larvae have well-developed external gills, which spread at the surface as the tadpole hangs vertically, and conspicuous lungs are present at a later larval stage. These two characters, along with the flotation of eggs, were considered to be adaptations to low oxygen concentrations of the environment. The temperature range in the natural habitat was 25 - 33° C.

Phyllomedusa hypochondrialis. -- This medium-sized frog is bright green on its dorsal surface in life. In preservation, this green color fades to slaty-blue. There are no indications of a green pigment in the skin or in other tissues of this species. Phyllomedusa

hypochondrialis was found on leaves of bushes and small trees at the edge of wooded areas. The large, yellow eggs of Phyllomedusa hypochondrialis are probably attached to leaves above standing water, into which the larvae drop after hatching. Movements of these pond-type tadpoles are rather slow.

Sphaenorhynchus aurantiacus. -- This small green hylid was collected from two similar localities in Surinam. In both habitats, the frogs called from cattails in standing water, 50 centimeters or more in depth. In a locality near Domberg, the frogs were found at an intersection of two drainage canals in which there was considerable aquatic vegetation. Along these canals were some shrubs and small trees. The second locality, south of Paramaribo, was a small cattail marsh, perhaps 30 meters in diameter and surrounded by forest except where it emptied into a roadside canal. Goin (1957) noted that all of the known species of this genus select areas with still waters as their breeding habitats, and that they call from the water, or from floating or emergent vegetation. Lutz (1954) stated that members of this genus lay eggs on leaves, but their development seems to be unknown.

Goin (1957) specifically noted that certain members of this genus have green bones (Table 1). From the description of color in other structures, it appears that green pigmentation of tissues is a general and striking characteristic of the genus Sphaenorhynchus.

Hyla dominicensis. -- Of the hylid species on Hispaniola, Hyla dominicensis is certainly the most common and has the widest distribution. It is very similar to Hyla brunnea, the most common and widespread of

Jamaican tree frogs, and to Hyla septentrionalis, the only tree frog of Cuba and surrounding islands. In the Dominican Republic, Mertens (1939) found Hyla dominicensis in all but the most arid habitats, from near sea level to more than 1000 meters in elevation. Barbour (1914) noted that the Museum of Comparative Zoology has many specimens from all parts of the island and Cochran (1941) noted that it is common in collections.

During October, 1965, I collected an adult and tadpoles of this species from Port-au-Prince and heard adults calling from trees in Petionville (400 meters elevation) and Cap Haitien. Adults were heard in May and August of 1966, most often during or after rain. Tadpoles were also collected during these visits. The first large chorus of this species which Mertens (1939) heard was on the evening of February 21 after the first hard rain. He noted breeding activity until September 29 and concluded that there is no definite breeding period. While Lynn (1958) collected adults and tadpoles of Hyla dominicensis in Haiti during mid-April, 1953, he did not hear them calling. He also noted that this frog produces a skin secretion which is irritating to cuts and scratches, and that the bones are green.

Mertens (1939) took note of the very rapid development of Hyla dominicensis tadpoles. He collected a number of these from a street puddle filled with rust-colored water on March 3. He stated that the larger tadpoles had a covering of grass-green algae on the back and only small hind limb buds, but developed into frogs in 10 days. Similarly, larvae that he collected from a cistern on February 21 had only rudimentary

hind limbs, but were in metamorphosis on March 4. Mertens found Hyla dominicensis in bromeliads in the coniferous forests, but did not state that they bred there. Noble (1923) indicated that he found tadpoles of this species at 2500 meters elevation in wheel ruts. Lynn (1958) found tadpoles of this species in a small pool at the head of a stream.

I found this species only in stagnant water: in a shaded pool in a dry stream bed, in a flooded wiregrass pasture exposed to the sun, in wheel ruts of a muddy road which were also exposed to the sun, and in concrete-lined pits which had been used for tanning hides. In the latter two habitats, the water contained so much solid material and algae that the tadpoles could not be seen except when they came to the surface to take air. The constant agitation of the water by the dozens or hundreds of tadpoles appeared to be largely responsible for the turbidity.

When the flooded pasture was visited in May, a large cluster of tadpoles was noted in one flooded corner. After tadpoles from the cluster came to the surface, they ordinarily returned to the group. This behavior continued until my presence caused the group to disperse. It is of particular interest since the temperature of the water was above 32° C. and the oxygen concentration averaged 0.7 parts per million. Brattstrom (1962) has shown that such aggregations of dark tadpoles absorb more radiant heat than do isolated individuals. He found water temperatures near such groups to be higher than water temperatures at a distance. He reasoned that the resultant higher body temperature caused an increase in metabolic rate, which decreased the time required for development.

After the group of tadpoles dispersed, the behavior of individuals was noted. Most appeared to be resting near the bottom of the flooded area while others appeared to be feeding along the bottom. At intervals, they came rapidly to the surface to gulp air and then returned to the bottom to rest or continue feeding. Four individuals which were observed for five or more intervals had average interval lengths of 17, 22, 27, and 31 seconds. It appeared that the smaller and more active ones surfaced more often. Several hours after some of these tadpoles had been collected, their tails appeared quite red. When this habitat was visited again in August, 1966, a flocculent precipitate covered most of the surface and no tadpoles were present.

Young larvae of Hyla dominicensis are black, but they become brown or gray and develop green pigmentation in bone and soft tissues during metamorphosis. The black pigmentation of the young may be an adaptation to an environment exposed to the sun, since this character is seen in Hyla vasta and Hyla septentrionalis in similarly exposed environments, but not in the Jamaican hylid tadpoles which develop in shaded habitats. Gills are well developed in this species of tadpole, but they appear to lack lungs until metamorphosis. Therefore, the air which they take at the surface must be kept in the mouth where gaseous diffusion takes place, and then the bubble is released at or near the surface prior to taking in a fresh gulp of air. The complex gill structure probably is important in filtering food, primarily algae, from the water. Like most vegetarian tadpoles, these have a long, coiled intestine. While the tail of this species is not particularly muscular,

it is similar to that of stream tadpoles in lacking the fin extension on the back.

Hyla septentrionalis. -- Of the native West Indian hylids, Hyla septentrionalis is the most widely distributed. This medium-sized tree frog is found throughout Cuba, where it is most abundant in the banana groves of the lowlands (Barbour and Ramsden, 1919). In addition it is common and perhaps has been introduced into the Cayman Islands, the Bahama Islands, the Florida Keys and southern Florida (Barbour, 1937). Barbour (1931, 1937) believed that this species was introduced into Key West on freight cars from Cuba. There are indications of subsequent introductions and range extensions in south Florida (King and Krakauer, 1966). Grant (1940) summarized references which indicated that this species was accidentally introduced into the Cayman Islands, but did not maintain itself on Cayman Brac. However, William Greenhood (personal communication) noted this species on Cayman Brac during the summer of 1965. Although there has been some question about its distribution on Grand Cayman, I have found it there at two localities--near North Side and just east of Boddentown--as well as in the Georgetown area. Specimens have been seen from all of the major islands of the Bahamas as far southeast as Acklin Island. A specimen of Hyla septentrionalis in the Institute of Jamaica was collected during the spring of 1965 at Highgate, St. Mary's Parish, Jamaica. This specimen resulted from the introduction of tadpoles from Hialeah, Florida, during the spring of 1961 (Edwin Todd, personal communication); there is no evidence that this species has bred in Jamaica.

Thus, the Cuban tree frog has become established in a number of localities through the activity of man, and its original range outside of Cuba would be difficult to determine. Part of the adaptability of this species may be related to its accommodation to human habitations; it is also found in mesophytic situations, but not in pineland or prairie habitats (Duellman and Schwartz, 1958; Barbour and Ramsden, 1919; Grant, 1940; Stejneger, 1905; Peterson, Garrell and Lantz, 1952; Neill, 1958).

During the present study, habitats of the Cuban tree frog were studied on Grand Cayman, on New Providence Island in the Bahamas, in the vicinity of Miami, Florida, and near Highgate, St. Mary's Parish, Jamaica. Cuba was not visited due to political restrictions. The two days' stay on New Providence, during May, 1966, was unproductive due to drought and a widespread lack of surface water.

In Miami, Florida, tadpoles of Hyla septentrionalis were found at a tropical fish hatchery and at the Serpentarium, a commercial enterprise concerned largely with snakes. Large larvae undergoing metamorphosis were noted in concrete tanks in both places. In addition, thousands of small tadpoles were found in the long, shallow, ornamental pool in front of the Serpentarium (Table 3, Habitat 21). Since this pool had been drained and cleaned just one week before, the tadpoles had hatched and grown to 5 millimeters in body length in less than a week. Although the water temperature of this pool was 33.9° C., the oxygen concentration was 9.2 parts per million. The pool had been drained to remove large amounts of algae, but the water appeared clear at the time of measurement; also,

the sky was overcast during much of the day so that it appeared unlikely that the high oxygen content was due to photosynthesis. At the time of measurement, a strong breeze was blowing and may have been responsible for the presence of so much oxygen in the shallow pool. Although the tadpoles were moderately active at the high temperature, they rose to the surface infrequently, and then as often to feed from the surface film as to take air.

The majority of my observations on Hyla septentrionalis were made during two visits to Grand Cayman--July 5-8, 1965 and October 28-30, 1965. At the time of the July visit, most of the island had been without rainfall for more than a month and was quite dry. Adult Hyla septentrionalis were found only in the vicinity of Georgetown; they became quite active following a shower on the evening of July 7. Tadpoles were not found in any of the cow wells inspected, but a number were collected from a small cistern which was well shaded. The cistern was located behind an abandoned church 6.4 kilometers southeast of Georgetown and was about 50 meters from the shoreline.

During the October visit, tadpoles were still present in this locality. Considerable rain had fallen during the two weeks prior to my visit so that a number of temporary pools of rain water had collected. Tadpoles were collected from several small woodland pools near South Sound, from a small shaded pond and from an open pond near North Side. The woodland pond and pools were less than 20 centimeters deep; the open pond was about 50 centimeters in depth.

The tadpoles and adults of Hyla septentrionalis are very similar to those of Hyla dominicensis; in fact, the two forms have been considered races of the same species (Barbour, 1937). The tadpoles of Hyla septentrionalis are black when young and become brown toward metamorphosis. They are primarily vegetarian, but on one occasion I observed a larger tadpole rapidly ingest a smaller one. The gills are well developed, but lungs are not. Body shape is rounded, and, like that of other hylid tadpoles in the West Indies, does not bear an extension of the tail fin. The rate of development is very rapid as previously mentioned. Grant (1940) stated that C. Bernard Lewis found "that eggs laid in a tiny puddle in coral or tree trunk during the night will be active tadpoles by mid-day." English (1912) found that these frogs breed any time of the year after a good rain, with eggs developing to frogs within a few weeks. He also indicated that the tadpole and frog are enemies of mosquitos which are abundant on Grand Cayman. This is probably true, since the smaller bodies of water in which these tadpoles are found are ordinarily clear and free of other obvious forms of animal life, such as mosquito larvae.

Hyla septentrionalis tadpoles have been found in brackish pools on the Florida Keys (Neill, 1958). Larvae of this species often have a clear envelope of fluid surrounding the body which is contained within an outer layer of skin. This would seem to be an adaptation which slows diffusion of water or salts between the tadpole body and its environment. It should also reduce heat exchange. It is possible that these tadpoles are capable of slight osmoregulation, since an individual lived nearly four days in sea water diluted by distilled water to 10 parts per thousand salinity. At 15 and 20 parts per thousand salinity, the

tadpoles died within three hours, but at 5 parts per thousand salinity, a tadpole lived for ten days before it was returned to fresh water. Since an isotonic solution for these tadpoles is presumably 7 parts per thousand, the first tadpole was able to maintain itself in a hypertonic solution for near four days.

A number of preserved young Hyla septentrionalis from Grand Cayman showed green bone pigmentation, as did a number of the larger specimens. Of more than 260 live adults collected from Miami, Florida, during June, 1966, none showed green pigmentation in bones; however, Clarence McCoy (personal communication) collected specimens with green bones from Homestead, Florida, during August, 1967. I was unable to determine more about the development of green pigmentation in this species because it did not undergo metamorphosis in the laboratory. I found the skin secretion of this species to be extremely irritating if rubbed into the eyes; it is also difficult to wash from the eyes.

Stream habitats

Flowing streams constitute the primary habitat of several species of tadpoles in the West Indies and South America. Temperature and oxygen concentration within these streams apparently have a narrow range of daily fluctuations. Streams inhabited by tadpoles were found to have temperatures between 20° C. and 25° C. in most cases. Since these streams were in hilly or mountainous areas, it seemed that the higher altitude was related to the relatively low water temperatures. Quite regularly, direct sunlight was blocked by clouds during the afternoon. These factors,

along with the shade provided by forest vegetation, probably account for stream temperatures remaining below air temperatures, which are also relatively low. Stream turbulence appears to keep oxygen concentration between 7 and 10 parts per million, but this is decreased where there is little turbulence and the stream moves more slowly (Table 4, Habitat 29). With a pH range of 6.0 - 8.6, the streams were similar to other surface waters, but were less acidic than water which collected in bromeliads. Dissolved solids demonstrated no definite trends.

Hyla maxima. -- This large, brownish species was found calling at Princess Irene Falls, Brownsberg, Surinam. Individuals called from tree sites 2 meters or more above ground. At least six individuals called from trees around the perimeter of the falls for three successive nights, even though there was no rain; none were heard elsewhere on the small mountain. Tadpoles in the pools at the base of the falls were believed to be of this species, as were eggs and approximately 50 small tadpoles found in a shallow rock basin (3 x 25 x 40 centimeters). The basin was in a rock ledge approximately 2 meters to one side of the main falls and 60 centimeters above the base of the falls. It received a steady supply of water from spray and droplets which fell into it. Since tall trees nearby surrounded the base of the falls, the basin remained in the shade except for about an hour at mid-day. Shortly after sunrise (7:35 A.M.), temperature of the basin water was 23.8° C. Near the end of the period of insolation (12:30 P.M.), the water temperature in the basin reached 29.9° C., while the air temperature was 28.1° C. Two hours later, the water temperature in the basin had dropped to 25.6° C.

Thus, water temperatures in the basin ranged from 23.8° C. to 29.9° C., while the temperature ofthe water in a pool at the base of the falls ranged from 23.4° C. to 25.5° C. Iron concentration in the basin was about three times as great as in the pool (Table 5). This may be important to young tadpoles, since it was noted that young Hyla brunnea tadpoles are more sensitive to iron deficiency than are the older ones. Concentrations of phosphate and pH were similar for both the basin and the stream pool. Oxygen concentration in the pool ranged from 8.3 to 8.9 parts per million; during the same period, the basin contained 2.0 to 3.1 parts per million of oxygen. Concentration of carbon dioxide in the basin shortly after it had been in the sun was 24.0 parts per million; at the same time, the concentration of carbon dioxide in the pool was 10.0 parts per million.

The eggs in the basin were of medium size--2 to 3 millimeters in diameter, not including the jelly--and rested on the detritus at the bottom of the basin. It was not known whether the eggs originally floated on the water surface. The yolk of the eggs was white rather than yellow and the animal hemisphere was darkly pigmented. Tadpoles in the basin were observed feeding on the eggs, which filled their alimentary tracts. They also came to the surface to take air, but do not appear to have lungs. When the water temperature was highest, these tadpoles were observed coming to the surface at intervals of 10 to 25 seconds.

None of the tadpoles observed showed green pigmentation. However, none had well-developed hind legs. The length of time required

for development was not determined. Three adult males were collected from this locality and all had green bones, soft tissues and plasma (Table 1).

Dowling (1960) found the tadpoles of this species in the Arima River of Trinidad. She found the jet black tadpoles--about 2 to 5 centimeters in length--swimming in schools. These dense aggregations of tadpoles, as many as 177 in a group, did not keep to sun or shade. Neither did the individuals in masses appear to feed; tadpoles observed feeding were scattered in shallow water.

Hyla vasta. -- Noble (1923) found this giant tree frog in the northern coastal mountains of Hispaniola and also at 2500 meters elevation in the central range which runs east to west. Mertens (1939) also found it in forested areas in the central range, but did not consider it exclusively montane, since he collected the species at 220 meters in one locality. It has been found by the present writer and others near Furcy in southeastern Haiti at 1350 meters elevation. In this unforested and agricultural area the adults take cover in crevices and under roots and sod which overhang the stream bank. I also heard a small chorus in a ravine near the Riviere Froid at an elevation of 200 meters.

The life history of the giant Hispaniolan tree frog was first recorded by Noble (1923). In the rain-soaked northern mountains of the Dominican Republic, he found Hyla vasta along the streams which flow through the rain forest. He stated: "After the sun has set, the giant tree frog, Hyla vasta, leaves his hiding place among the tree tops and descends to some rocky ravine. There flattened out on a mossy boulder

in midstream, he rests for hours, seemingly enjoying the cool mists which arise from the torrents. So closely does the frog resemble the moss and lichen of his surroundings that he would rarely be observed were it not for his big shiny eyes, which are conspicuous even when closed, the lower eyelid being translucent"(p. 56).

While Noble (1923) found that the skin secretion of this species caused inflammation and irritation to his hands, I did not find this to be true of the frogs collected from Furcy. Neither the boys who caught them, nor I, after handling them, experienced any discomfort.

Noble (1927) described the early development and habits of the young as follows: "Hyla vasta laid its eggs in little basins in the gravel and stones on the edge of the pools in the mountain torrent (one observation). Six days after hatching, the larvae made their way out of one of the basins over wet stones into the torrent pool. As they grew older they developed better stream lines than the tadpoles of Hyla dominicensis. They were equipped with more rows of teeth. The mouth was larger and better adapted to holding on to rocks in the stream. Its tail was thicker and more muscular than that of the stagnant-pool tadpole"(p. 108).

He also reported that the eggs of Hyla vasta are pigmented and are stuck to rocks at the bottom of the basin, and that they give rise to dark tadpoles with small external gills, which do not rise to the surface. The temperature of the stream he recorded as 21.7 - 25.6° C., averaging 23.7° C.

My observations near Furcy were similar, except that I was unable to find a basin in which the young were developing. Half-grown to metamorphosing larvae were found at the bottoms of pools. The mottled gray pattern of the larger tadpoles makes them rather obvious against the silt on the pool bottoms. Occasionally these larvae will move under stones in the stream bed or attach themselves to rocks by means of their large sucking mouths. The sucking mouth is important for stabilizing the tadpole in the stream current and also for scraping plant food from rock surfaces. When disturbed, they may allow themselves to be swept downstream by the current.

Like Hyla dominicensis tadpoles, the larvae of Hyla vasta have well-developed gills. In the stream habitat, these gills seem to serve them well, since they were never observed taking air from the surface. In the laboratory, six members of this species were kept in a graduated cylinder which contained water with 0.5 parts per million of oxygen. At first, the tadpoles rested at the bottom when not surfacing for air, but after several hours they remained near the surface and were inactive except for taking air.

Green pigmentation appears in the large tadpoles at about the beginning of metamorphosis. The bones of metamorphosed young are green and this color is present in some of the large adult individuals. Slightly older frogs found among rocks along the stream also had green bones.

Hyla heilprini. -- This medium-sized species of Hispaniola was first seen by Noble (1923) as a brightly colored male which gave its shriek while sitting on a rock in the middle of a torrent in the northern

mountains. Although he did not capture that individual, he obtained tadpoles nearby. In cold streams above 2500 meters in the central regions he heard again the loud call of Hyla heilprini above the roar of the cascades. While the average temperature in the first stream was 24.7° C., it was 12.6° C. in the second locality at the higher elevation. Noble found the tadpole of Hyla heilprini to be more streamlined than the previous two species and considered this an adaptation to living in torrents, rather than in slower moving streams or puddles. Since these larvae were obviously adapted to an environment with a high oxygen concentration, Noble had doubts about being able to maintain them in captivity, but he was successful in raising them through metamorphosis.

From Noble's (1923) observations in the Dominican Republic, specimens from the Museum of Comparative Zoology from several localities along the southern peninsula of Haiti, and personal observations, it appears that Hyla heilprini is widespread on Hispaniola. I believe that I heard the species just west of Port-au-Prince in a ravine near the Riviere Froid, less than 200 meters above sea level. It would seem that wooded ravines are more important to this species than are high elevations. Cascading streams probably are necessary for its reproduction, as both Noble (1923) and I found its tadpoles only near waterfalls.

Green pigmentation was present in all of the dozen or more tadpoles which I collected. These were well-developed tadpoles, with either two or four legs, which were olive green with black spots dorsally, blue-green and white ventrally, and the distal portions of the limbs were orange in color. Since only the distal portions of the limbs were orange,

one wonders if this was the result of extreme effects of temperature or oxygen upon a pigment which was more generally distributed through the body. An adult of this species collected from a banana stalk was bright grass-green on the dorsal surface with white and blue ventrally and blue and yellow on the flanks. Later the back became quite dark. This specimen had green bones and green plasma. Noble (1923) described the dorsal color of a living specimen as "golden green."

Hyla pulchrilineata. -- Except for its light-colored longitudinal stripes, lack of an interocular bar, and larger size, this small green tree frog of Hispaniola is quite like Hyla wilderi of Jamaica. It has been collected from widely scattered points on the island and Cochran (1941) noted that it seems to be common in certain localities. From the records of Noble (1923), Cochran (1941), and Lynn (1958), it seems likely that this species has a distribution similar to those of Hyla vasta and Hyla heilprini: in forests at higher altitudes and in wooded ravines at lower altitudes. This species appears to be the least common of the four hylids of Hispaniola; I did not encounter adults or tadpoles during my visits to Haiti. Noble (1923) and Lynn (1958) found adults calling from leaves in the vicinity of streams. Lynn (1958) described its call as a faint clicking like a small telegraph instrument, similar to that of Hyla wilderi.

Noble (1927) stated that Hyla pulchrilineata young develop in water as do the other hylas on the island, but apparently he has given no further details. There are no known specimens of Hyla pulchrilineata tadpoles in the major museums of eastern North America (including the

American Museum of Natural History where Noble deposited most of his specimens). Dr. Lynn and Dr. Albert Schwartz (personal communications), both of whom have collected in this area, are unaware of any tadpoles of this species which have been collected. It seems best to consider Noble's statement as a supposition on his part.

The color of the bones of this species has not been recorded.

Bufo marinus. -- This very large toad was found breeding in several habitats including temporary pools in Surinam and near drainage canals and tropical fish hatcheries in Miami, Florida. It has been introduced into Jamaica, where it appears to be the only anuran species which breeds in ponds or slow-moving portions of streams. Neither young, which are vegetarian, nor adults are known to have green tissues under normal circumstances.

Leptodactylus albilabrus. -- Tadpoles of this Puerto Rican species were collected from a roadside stream on El Yunke, the highest mountain on the island. Water flowed rapidly in the stream, thus accounting for its high oxygen concentration (Table 4). Both large and small tadpoles of this species came to the surface, evidently to take air, but their lungs do not appear to be developed at these stages. This species feeds on vegetation and does not develop green pigmentation as it undergoes metamorphosis.

Resume of Ecology and Breeding Habits

Considering the great variety of aquatic habitats available in the Neotropical Region, it seems that anurans have taken advantage of most of them. The species with green pigmentation are well

distributed throughout the Neotropical Region, but appear to be restricted to certain habitats or families of frogs. As Barrio (1965[a]) noted, green pigmentation is found in only three families of South American frogs. Hylids and centrolenids are primarily arboreal following metamorphosis, the period of life when they appear most likely to have green pigmentation. Pseudids are generally considered to be highly aquatic as adults. Although I found relatively little green pigment in some adult Pseudis paradoxus paradoxus in Surinam, Barrio (1965[a]) found high concentrations of biliverdin in Pseudis paradoxus platensis, Lysapsus limellus and Lysapsus mantidactylus adults. While green pigmentation appears in tree frog tadpoles with metamorphosis, it preceded metamorphosis by an extensive period in Pseudis paradoxus paradoxus. Thus, the pseudids differ from hylids and centrolenids in both the type of habitat and in the time of onset of pigment development. It seems logical to assume that the hyperbiliverdinemia of pseudids has a cause which is different from that of the two tree frog families. In addition, the point should be made that terrestrial and fossorial anurans--toads largely--have not been reported to have biliverdin in their tissues.

Since distributional and ecological factors have not yielded a satisfactory explanation for the accumulation of green pigment, it appears logical to turn to physiological studies.

III. PHYSIOLOGICAL FACTORS RELATED TO CHLOROSIS

Previous studies have shown that the immediate cause of chlorosis is the production of biliverdin in quantities greater than those which are excreted. It has not yet been determined if chlorosis in frogs is due to a high rate of biliverdin formation, impairment of bile pigment excretion, or both.

Since hemoglobin of the blood is ordinarily the main source of biliverdin, it is logical that one should study this protein and the red blood cells in which it is found. If chlorosis is due primarily to a high rate of biliverdin formation, one might expect to find higher concentrations of hemoglobin or higher rates of hemolysis in chlorotic frogs than in others. In addition to chlorosis itself, high rates of hemolysis might be indicated by low red cell counts, or by blood smears which show immature erythrocytes or cell fragments. Should hemolysis or other process be shown to be the cause of increased biliverdin, then the cause of such a process should be determined. In the case of rates of hemolysis, it is known that they may be correlated with temperature, concentrations of chemical agents, or differences in red cell structure.

Impairment of bile pigment excretion would involve the liver and its associated ducts. Blockage of extra-hepatic ducts is readily determined by the absence of biliverdin from feces. Blockage or restriction of bile flow within the liver may be difficult to detect,

but histological study ordinarily shows the degeneration of liver which is associated with prolonged cholestasis.

Several factors indicate that the study of tadpole respiration may shed light upon chlorosis. First of all, the present study has shown that green pigmentation first appears in the tadpole stage of several species (Table 2). Secondly, the low oxygen tensions of some tadpole environments suggest that the larval stages have special respiratory adaptations or that tissue damage may result from oxygen deficiency. The degradation of respiratory pigments--apparently the immediate cause of chlorosis--suggests a disruption or decrease of oxygen transport within the organism. Since the liver is quite sensitive to oxygen deficiency, one wonders if the preceding factors are concerned with possible liver malfunctions. Finally one should consider the high environmental temperatures to which these organisms are exposed, and the greater stress which this factor places upon respiratory and hepatic systems.

In order to determine the importance of biliverdin formation to chlorosis, several attempts were made to induce green pigmentation in vivo, using non-chlorotic species. This was followed by a broader physiological study of Neotropical frog species--both chlorotic and non-chlorotic--to clarify the importance of biliverdin formation, liver function, and other factors which might pertain to the green pigmentation.

Attempts to Reproduce Green Pigmentation In Vivo

During the early part of the study, attempts were made to duplicate the high concentration of tissue biliverdin, particularly as it occurred in bone. These experiments were carried out upon species which

were readily available in Florida, none of which had green bones. Four methods were utilized in attempts to increase the concentrations of biliverdin in the experimental animals. Phenylhydrazine hydrochloride was used to destroy the red blood cells in the frog, thus causing its hemoglobin to be degraded into biliverdin more rapidly. The second method was the injection of additional hemoglobin, presumably of mammalian origin, again to increase the rate of biliverdin formation. Third was the direct injection of biliverdin into the animal. The fourth method was to induce an iron-deficiency anemia, such as that known to cause chlorosis in man.

Phenylhydrazine hydrochloride administration

Since biliverdin formation was induced in Bufo arenarum by means of phenylhydrazine injection (Cabello, 1943), this method was used in an attempt to develop green bones in species which were known not to have green bones.

Two adult specimens of Bufo terrestris, along with ten Hyla cinerea, were given intraperitoneal injections of phenylhydrazine hydrochloride in water in October 28, 1964. The dose was at the rate of 1 microgram of phenylhydrazine hydrochloride per gram of body weight of the frog. On November 19, 1964, each of these frogs was given a second dose of 2 micrograms of phenylhydrazine hydrochloride per gram of body weight. During the period of the experiment these frogs were fed twice a week. The animals were observed daily until December 9.

Injection of phenylhydrazine hydrochloride caused destruction of red blood cells and formation of biliverdin, but like the other

methods used, it did not induce detectable hyperbiliverdinemia. Severe anemia developed in specimens of Hyla cinerea as a result of injection of this drug. Two smaller individuals died several days after the second injection. Post-mortem examination showed that very few red cells were present in the blood vessels or elsewhere. There was slight indication of hemopoietic activity in the long bones; the spleen was pale and showed no increase in size. The kidneys were slight yellow in color, rather than the usual red. The frogs appeared emaciated. The liver was normal in the larger frog examined; the smaller frog had an enlarged gall bladder filled with green fluid and its liver was smaller than expected. None of the frogs and toads used in this experiment showed any evidence of green pigmentation in bone, blood or other tissues outside of the digestive tract.

Administration of hemoglobin solution

A second attempt to increase the amount of biliverdin in living anurans involved the intraperitoneal injection of a 1 per cent hemoglobin solution. Specimens of Hyla septentrionalis (eleven individuals), Rana pipiens (four individuals), Rana catesbeiana (two individuals), Hyla gratiosa (one individual), Bufo terrestris (two individuals), and Hyla cinerea (seven individuals) were injected with either 1 per cent hemoglobin in phosphate buffer, or with phosphate buffer (pH 7.4) alone, or were given no treatment. Fluids voided by these animals were taken from the jars in which they were kept and analyzed with the Beckman DU spectrophotometer.

Intraperitoneal injection of hemoglobin solution resulted in no clear indication of green tissues in any of the species. Several individuals voided substances which had absorption peaks that corresponded to biliverdin or hemoglobin. There appeared to be no addition of green pigment to the tissues. Judging from the color of the fluids voided by these animals, bile pigments were formed and eliminated within twenty-four hours after injection of hemoglobin. However, it should be pointed out that green pigment was not excreted by all of the frogs which were injected with hemoglobin. In addition, one untreated specimen and one injected with only phosphate buffer demonstrated green pigment in voided fluids. This was also true of other untreated frogs in the laboratory.

Injection of biliverdin

Still a third method was used--the direct injection of biliverdin in sesame oil or phosphate buffer (pH 7.4). On one occasion, an attempt was made to mobilize bone calcium prior to injection of biliverdin. Several specimens of Hyla cinerea were injected with 0.5 (.005 cc) units of Lilly's Parathyroid Extract per gram of body weight. The same dosage was repeated twelve hours later. After another six hours, an intraperitoneal injection of biliverdin in sesame oil was administered. Finally, they were given two drops of cod liver oil after an hour. The latter step was intended to stabilize calcium in bone because of its vitamin D content (Cantarow and Schepartz, 1957).

Biliverdin solutions injected into the peritoneal cavity caused no apparent change in the color or other visual characteristics

of the tissues. This treatment was discontinued after a relatively short period of time because it did not appear practical to continue. Attempts to decalcify bone or otherwise change its pigmentation *in vivo* were unsuccessful.

Induction of iron deficiency anemia in tadpoles

Iron deficiency is the most common cause of anemia in man and may become pronounced during pregnancy or during periods of growth. In one of its more serious forms (chlorosis) iron deficiency anemia causes the skin to become green. During the nineteenth century, this was found regularly among girls and young women, particularly those with menstrual disorders.

Little is known about iron metabolism of frogs. Iron is absorbed in the duodenal region of the intestine and incorporated into eggs and hemoglobin (Brown, 1964). However, it seemed possible that a lack of iron could be a cause of green pigmentation in frogs, as it is in man. The main difficulty in running an experiment to test this idea in frogs or most tadpoles is that their intake of iron cannot be regulated easily. Live insects constitute the normal diet of frogs, and most tadpoles feed upon plant life and other organisms. Tadpoles of *Hyla brunnea* are different in this respect, since they feed upon frog eggs, usually those of their own species. These tadpoles develop normally when they are fed Gerber's strained egg yolk, and kept in spring water. The strained egg yolks were known to contain 3.07 milligrams of iron per hundred grams (Gerber Products Company, 1965). It was thought that the high concentration of phosphates in egg yolk (290 milligrams of

phosphorus per hundred grams) would decrease iron absorption, due to the poor absorption of iron complexed with phosphates (Cantarow and Schepartz, 1957). The animals were kept in glass-distilled water, rather than spring water, in order to reduce the iron concentration further.

Two matched groups of twelve tadpoles were placed in small glass bowls which had been acid-cleaned, rinsed and filled with glass-distilled water. During the course of the experiment, the water was changed twice a week and fresh egg yolk was added immediately thereafter. An excess of food was always available to both groups. The only difference in treatment between the two groups was that ferrous sulfate (reagent grade) solution was added to the water of the first group. Individuals of the first group appeared to develop normally, as did the four larger individuals of the second group. Of the eight smaller tadpoles of the second group, all became pale yellow in color and five were dead after two weeks. At this time, ferrous sulfate was added to the water of the second group. After three days, the second group of tadpoles became the same light brown color as those of the first group. The darker pigmentation appeared to be the result of an increase in hemoglobin. These observations were taken to indicate that limitation of iron intake of rapidly growing tadpoles resulted in an anemia, which was relieved by addition of more iron. It is also possible that trace elements such as copper or cobalt may have been responsible for hemoglobin formation in both groups. In either case, the anemia did not cause development of green pigment in the skin of any of the tadpoles.

Physiological Characteristics of Chlorotic and Non-Chlorotic Frogs

This portion of the study was directed toward finding physiological differences between chlorotic and non-chlorotic frogs. Frog blood, tadpole respiratory rates, and histological sections of liver were the main subjects of study.

Methods of study

Hemoglobin determinations. -- The amounts of hemoglobin present in anuran blood was determined by the acid-hematin test (Cohen and Smith, 1919). A Heilige hemoglobinometer was used for this purpose, since much of the work was done under field conditions.

Blood was collected from a large blood vessel or the heart into heparinized capillary tubes. From these, 20 microliter samples of blood were transferred to a graduated test tube which contained $\frac{1}{2}$ milliliter of 1 per cent hydrochloric acid solution. After mixing the contents of the tube, they were allowed to stand for ten minutes in order to insure complete hemolysis of the red blood cells. Then distilled water was added until the color of the solution matched the color standard of the hemoglobinometer. The calibration mark at the meniscus of the fluid indicated the hemoglobin concentration of the blood in grams per hundred milliliters.

Red blood cell counts. -- Blood samples obtained as for hemoglobin determinations were diluted and counted by standard techniques using a Spencer Bright Line hemocytometer. Four counts were made for each individual and the average was used as the red blood cell count.

Red blood cell measurements. -- An optical micrometer was used to measure length and width of twenty-five red blood cells from each individual. Measurements were made from smears prepared and stained in the field. Average cell length and width were determined and converted to microns. These blood smears were used to study abnormal cell types in the blood.

Effects of temperature. -- In order to determine the effect of temperature upon frog erythrocytes, blood from specimens of Hyla squirrella, Bufo terrestris and Scaphiopus holbrooki was collected in heparinized capillary tubes. The tubes were plugged with clay at both ends and kept in a water bath at 45° C. for varying periods of time. After this treatment, smears of the blood were made, stained and studied.

Measurement of tadpole respiratory rates

Measurement of respiratory rates of tadpoles was carried out by two different methods. During the earlier part of the work, oxygen uptake was determined by use of Warburg constant volume respirometers as described by Umbreit, Burriss and Stauffer (1964).

The second method was improvised in order to determine the rate of oxygen utilization by tadpoles shortly after capture, under either controlled or field conditions. Necessary apparatus for field measurements were Erlenmeyer flasks with rubber stoppers, a Precision Scientific portable oxygen analyzer, and a wristwatch. The simple technique consisted of the following steps:

1. Clean 125 ml Erlenmeyer flasks were calibrated to determine their actual volume when stoppered.
2. The appropriate number of clean Erlenmeyer flasks were filled with clean tap water of the desired temperature.

3. The probe of the oxygen analyzer was calibrated in air. After dipping the tip of the probe into clean water, it was waved in the air while two ammeter readings were taken. The ammeter of the oxygen analyzer measures electrical current between silver and lead electrodes located in the tip of probe; this current is directly proportional to the oxygen which diffuses through the covering membrane and into the space between the electrodes. Air temperature was determined at the same time by use of a thermistor attached to the probe. These data were used to calculate the sensitivity of the probe under prevailing conditions.

4. The probe of the oxygen analyzer (which had been taped to fit snugly into the neck of the Erlenmeyer flask) was lowered carefully into the neck of the flask until the probe formed a watertight seal with the neck of the flask. After this was accomplished, the tip of the probe projected slightly below the neck of the flask. When done properly, no bubbles were trapped in the flask.

5. While holding the flask and probe together, they were rotated vertically through a 90 degree arc, so that their axis was in a horizontal plane. From this position, the flask was shaken from side to side in order to cause water to flow past the tip of the probe. This procedure was necessary to improve the accuracy of the method. In the laboratory, a magnetic stirrer was used to cause a flow across the tip of the probe.

6. Once the indicator of the ammeter became steady, a reading was made. The ammeter was then turned off for a short time and then turned on again. While the shaking continued a second reading was made and the average of the two readings was recorded. Probe sensitivity, ammeter reading and water temperature was used to calculate the initial oxygen concentration.

7. Once the data necessary to determine the initial oxygen concentration were obtained, a single tadpole was introduced into the flask, and the flask was stoppered. If an air bubble was trapped within the flask, sufficient water from the original source was added so that the flask was completely filled with water when stoppered. This was necessary to prevent intake of air by the tadpoles and also to prevent immeasurable exchange of gases between air and water. Note was made of the time that the flask was stoppered.

8. After an appropriate length of time, the flask was opened and the final oxygen concentration determined in the same manner as the initial oxygen concentration (steps 3 through 6).

9. The tadpoles were fixed in formalin and each individual was labeled so that their weights could be measured upon return to the laboratory. Since formalin preserved tadpoles were found to lose nearly 10 per cent of their original weight, the weight of each preserved tadpole was multipled by the conversion factor 1.094 to obtain a more accurate estimate of the original wet weight.

10. Since the difference between initial and final oxygen concentrations was given in mg/l, it was necessary to make additional calculations to obtain the respiratory rates in terms of μl O_2/g/hr. This was achieved by use of the following equation:

$$\text{Respiratory rate } (\mu l\ O_2/g/hr) = \frac{\text{Change in } O_2 \text{ concentration (mg/l)} \cdot \text{Water volume } (\mu l)}{1.429 \text{ (conversion to ml of } O_2) \cdot \text{Tadpole weight (g)} \cdot \text{Time (hrs)}}$$

Although the error incurred in this method approached 3 per cent or even 5 per cent under some circumstances, the necessary equipment was not nearly so cumbersome to transport as manometers and pipettes; cleaning was not a problem, nor was the equipment as likely to be damaged in transit. In addition, if one desired to maintain the animals at a specific temperature during an experiment, a wash basin or large pan could hold a dozen or more flasks at one time and the temperature of the water in the bath could be maintained within 1° C. of the desired temperature by addition of ice or warmer water, as required; hence, no special bath, refrigerator or heater was required. In addition to technical advantages of the method, it allowed measurement of oxygen uptake under actual field conditions, although this opportunity was not realized. Since it was possible to make measurements shortly after the tadpoles were collected,

there was little time for them to become acclimated to different environmental conditions. With modifications in the size of the flask and diameter of probe, larger organisms could be studied.

Aside from the lower accuracy of the measurement of oxygen change, disadvantages in relation to manometric techniques included: slightly more variation in temperature due to lack of automatic controls and constant stirring; the practical impossibility of beginning and ending an entire series simultaneously, or of taking readings at regular intervals during the experiment.

This technique was developed in the laboratory at Gainesville, where it was used to measure oxygen uptake of Hyla dominicensis, Hyla brunnea, Hyla vasta, and Leptodactylus albilabrus maintained at 25° C. in a water bath. Respiratory rates of Hyla maxima and Pseudis paradoxus were studied at 25° C. in Surinam; similarly oxygen uptake of Hyla vasta, Hyla dominicensis and Hyla heilprini were determined at 25° C. in Haiti.

Liver tissue sections. -- Blocks of frog liver tissue were fixed in Bouin's fluid and then changed to 50 per cent ethanol for storage and transport back to the laboratory. These tissues were embedded, sectioned and stained with hematoxylin and eosin by standard techniques.

Results

Hemoglobin concentrations and red cell counts. -- The small green-boned species, Hyla punctata and Sphaenorhynchus aurantiacus, had the lowest concentrations of hemoglobin of the species studied. Another small form with green tissues, Hyla wilderi, along with Sphaenorhynchus aurantiacus, had the lowest red blood cell counts. Hyla punctata had the

lowest concentration of hemoglobin per cell, along with an individual of Phrynohyas venulosa. Yet species with maximal values in these categories also have green tissues. These include Hyla maxima and Phrynohyas venulosa. However, the species which has the largest amount of hemoglobin per cell, Phyllomedusa hypochondrialis, does not have green tissues. This is evidently a reflection of the large size of the red blood cells in this species (Table 7). There appears to be no correlation between the blood characteristics studied and the presence of green pigmentation.

It should be noted that heparinized blood of Pseudis paradoxus tadpoles and Sphaenorhynchus aurantiacus males completely hemolyzed after being kept in their own blood plasma at room temperature overnight. This was not noted in other species with green tissues or in those without green tissues.

Red cell size. -- From Table 7, it can be seen that cell lengths range from 12.8 to 21.6 microns. Cell widths vary from 9.9 to 14.5 microns. To obtain an approximation of erythrocyte volume for each species, the average cell length was multiplied by the average width. The largest cells are those of Phyllomedusa hypochondrialis (20.6 x 14.5 microns) and Sphaenorhynchus aurantiacus (21.6 x 13.9 microns). Smallest cells were those of Pseudis paradoxus (13.2 x 12.0 microns), Phrynohyas venulosa (12.8 x 10.8 microns) and Osteocephalus taurinus (13.1 x 10.8 microns). The most variable cells were those of Hyla punctata. All of these species except Phyllomedusa hypochondrialis are chlorotic.

Examination of blood smears. -- Blood smears stained with Wright's stain revealed certain characteristics which may or may not be

related to green pigmentation (Figures 12-16). A list of these and some others, according to the organisms in which they were observed, follows:

Pseudidae
Pseudis paradoxus -- adult male - Cells of this individual tended to form cytoplasmic blebs; tadpole blood hemolyzed upon standing overnight.

Bufonidae
Bufo typhonius -- adult - an erythroplastid was observed in the blood of this individual.

Atelopodidae
Atelopus sp. -- male - erythrocytes showed coagulated cytoplasm.

Hylidae
Hyla boesemani -- adult - cells have coagulated cytoplasm, as occurs after slight heating.

Hyla crepitans -- adult - most cells hemolyzed.

Hyla eqleri -- male - cells not unusual.

Hyla geographica -- adult male - nothing unusual.

Hyla lanciformis -- adult - most of the cells were hemolyzed.

Hyla leucophyllata -- about half of erythrocytes have coagulated cytoplasm.

Hyla minuta -- adult - most of the cells have coagulated cytoplasm as occurs on heating.

Hyla misera -- some cells have odd shapes.

Hyla maxima -- adult - many lymphocytes present.

Hyla punctata -- adult - cell membranes are irregular; green cytoplasmic and extracellular granules present on stained slides. Erythrocytes appear immature.

Hyla rubra -- adult - nothing unusual.

Osteocephalus taurinus -- adult - many erythrocytes with vacuolar nuclei.

Phrynohyas venulosa -- red cell cytoplasm coagulated.

Phyllomedusa bicolor -- appears to have many leukocytes.

Phyllomedusa hypochondrialis -- erythrocytes are basophilic.

Sphaenorhynchus aurantiacus -- cytoplasm of erythrocytes appears vacuolated, with greenish refractile material in vacuoles (stained slide). Blood of three males hemolyzed overnight.

Leptodactylidae

Leptodactylus pentadactylus -- some red cells show coagulated cytoplasm.

Effect of temperature upon frog red cells. -- Slight changes in a few cells were noted after ten minutes at 45° C. After four hours at 45° C., cytoplasm had coagulated in nearly all of the red cells of Hyla squirrella and Bufo terrestris (Figures 9-11); the red cells of Scaphiopus holbrooki were abnormally shaped after this time, but the cytoplasm had not coagulated.

Respiratory rates of tadpoles. -- The respiratory rates of tadpoles and froglets of green boned species (Tables 8-10) are similar to those previously recorded for amphibian larvae (Hopkins and Handford, 1943). The basis of recording respiratory rate in the study was the uptake of microliters of oxygen per gram of wet weight of tadpole per hour. In order to convert this to an approximate dry weight basis, the rate of oxygen uptake should be multiplied by eight. Size range of the tadpoles in the present study is much greater than in previous ones, but it can be seen that respiratory rate is a function of tadpole size and that the species is not very important in this respect (Figure 8). As is to be expected, respiratory rates increase with increasing temperature (Figure 7).

It should be noted that the respiratory rate of Hyla brunnea tends to drop sharply between 35 and 40° C. (Table 8, Group A), whereas the respiratory rate of Hyla septentrionalis does not (Table 8, Group C). When the Hyla septentrionalis tadpoles were removed from the flasks, they appeared to be in good condition (heart rate was about three beats per second). On the other hand, these Hyla brunnea tadpoles were limp after two hours at 40° C., and one did not recover. This is an indication that the critical thermal maximum of Hyla brunnea larvae is lower than that of the Hyla septentrionalis tadpoles. This might be expected when one considers the higher temperatures to which Hyla septentrionalis tadpoles are often exposed (Table 4). These higher temperatures are largely due to the lower elevation and greater insolation of Hyla septentrionalis habitats. Hyla brunnea is more often found in shaded areas and at greater elevations than are available to Hyla septentrionalis over most of its range.

Along these lines, it may be noted that the froglet of Hyla lichenata appeared in poor condition after being kept at 35° C. for less than two hours (Table 10). It would appear that Hyla lichenata is more sensitive to this temperature than is Hyla brunnea at the same stage. This may account for the fact that while Hyla brunnea is found throughout the range of Hyla lichenata, the former species extends its range to lower elevations and more exposed habitats than the latter.

Tadpoles of Hyla brunnea kept in the laboratory were exposed to low water temperatures during the early morning of November 30, 1965. This caused some mortality and the surviving tadpoles appeared pale,

similar to those which had iron deficiency. Very little movement of the tadpoles was evident when the water temperature in their containers was 16.8° C. Lynn (1940) suggested that the lack of large bromeliads above 1600 meters in the Blue Mountains may be the reason for Hyla brunnea not being found at such altitudes. Low temperatures at these altitudes may also be a factor which prevents this species from moving to higher elevations.

Liver histology. -- Study of liver sections of ten species of frogs from Surinam is of interest (Figures 17-20). No dead or completely degenerated tissue was seen in these slides but there were several indications of conditions or activities which may cause changes in bile excretion. There did not appear to be any obstructions to the bile duct or gall bladder in the majority of individuals examined, but those with green tissues and plasma usually had very concentrated bile in the gall bladder, judging from its dark blue color. A resume of liver histological characteristics follows:

Pseudidae

Pseudis paradoxus -- adult male - liver apparently normal except for very heavy pigmentation (the liver appeared black before dissection);
-- adult female - as in male, with possible cell fragments in blood of liver;
-- tadpole - liver structure apparently normal; much less pigmentation than in adults; cell fragments in blood of the liver; many immature erythrocytes present in circulating blood.

Atelopodidae

Atelopus sp. -- male - some liver cells appear enlarged and have rarified cytoplasm; some pyknotic nuclei present; otherwise apparently normal.

Hylidae

Hyla geographica -- adult male - veins of liver partly occluded; considerable pigment present in liver cells.

Hyla leucophyllata -- adult female - liver apparently normal; contains many erythrocytes.

Hyla maxima -- adult male - fat deposition taking place in one area; erythrocytes appear to be undergoing disintegration in liver; relatively little pigment present.

Hyla minuta -- adult male - some irregularly shaped nuclei; considerable pigment present.

Hyla punctata -- adult male - many nuclei present in liver tissue; these are quite variable in size, shape and staining characteristics; some may be pyknotic; very little pigment is present in the liver; many unusual cells are present in blood, perhaps immature erythrocytes.

Osteocephalus taurinus -- adult female - liver pigmentation less than normal; odd-shaped nuclei present in liver.

Disease as a Cause of Chlorosis in Frogs

Early in this study, it was assumed that disease was not principally responsible for the green pigmentation of frogs. Since several writers had accepted green bones or other green tissues as traits which were characteristic of certain species, it was not logical to believe that a bacterial or viral disease would affect all the members of a species or population in a nearly uniform manner. Neither was it logical that a genetic disease should be passed from generation to generation with such regularity. Thus, it was tentatively assumed that the normal metabolism of chlorotic frogs was responsible for their green pigmentation.

On the other hand, disease cannot be ruled out as a possible cause of biliverdin accumulation. Individual adult specimens of Hyla septentrionalis and Hyla dominicensis may have dark green, light green or white bones. Here is an example of what one might expect if some of the frogs were diseased, but such individual variation may also have other causes.

A better example of the effect of disease might be demonstrated by a specimen of Osteocephalus taurinus which arrived in the laboratory shortly after its death. The individual had been kept in another laboratory for several months, but did not eat during the latter part of its stay there. Dissection showed the internal organs to be darker than in most frogs. Blood vessels were a bluish-purple rather than red in color. An olive-green fluid was found in the coelomic cavity, while the dorsal lymph sac was nearly filled with a clear, viscous, blue-green fluid. The gall bladder was filled and appeared dark blue externally, but contained a clear fluid and a solid brown substance, perhaps bile salts. The dark color of the internal organs was due primarily to the presence of biliverdin, which appeared to be in most of the tissues, including bone. A cross-section of femur showed that the green pigment was found throughout the bone, but that alternating concentric lamellae of light and dark green were present. Microscopic examination showed that the liver cells had completely degenerated. A histochemical test for liver glycogen was made by means of the Periodic Acid-Schiff reaction. Aggregations of dark pigment were the only structures which were PAS-positive. These were believed to be a lipofuscin pigment from the degeneration of red blood cells. Unlike melanin, lipofuscin is PAS-positive (Dubin, 1958). These liver sections appeared similar to photographs of liver in acute viral hepatitis published by Popper and Schaffner (1957, p. 437). While the actual cause cannot be pinpointed, an obvious pathological condition of the liver prevented the normal excretion of bile pigment and evidently caused it to pass into the lymph.

DISCUSSION

Earlier in this paper, evidence was presented to show that chlorosis is the result of the accumulation of biliverdin in the tissues. Therefore, this condition corresponds to human jaundice, which is due to accumulation of bilirubin. Presumably, the obscure causes of chlorosis would parallel those of jaundice, which were recently outlined by Williams (1965).

There appear to be four basic causes of jaundice. Abnormally high rates of hemolysis cause an increase of bile pigment which may exceed the liver's capacity of excretion. Defective absorption or conjugation of bile pigment by the hepatic cells, or obstruction of the various biliary ducts can cause jaundice. These basic causes of bile pigment accumulation will be considered in relation to chlorosis of frogs.

High Rate of Red Cell Hemolysis

High rates of hemolysis and bile pigment formation might be expected in frogs which have a high rate of red cell replacement because of high erythrocyte counts or short red cell life spans. From Table 6, one notes that there are chlorotic species with high (Phrynohyas venulosa), low (Hyla wilderi), and intermediate red cell counts. Species which lack the green pigmentation demonstrate a similar range of red cell counts. Little information regarding frog erythrocyte survival times is available, but my unpublished data on this subject suggest that there is no significant

difference between red cell survival times of chlorotic and achlorotic frogs.

Blood smears from adults of Hyla crepitans, Hyla lanciformis, Sphaenorhynchus aurantiacus and a tadpole of Pseudis paradoxus have demonstrated that the majority of erythrocytes of these individuals were hemolyzed within a few hours after the samples were taken. The changes in cell structure or shape which preceded hemolysis are not known. Likewise the actual cause of hemolysis is unknown. Since unconjugated bilirubin is known to cause hemolysis in vitro (Cheung et al., 1966), it seems likely that plasma biliverdin induced hemolysis in the species listed above, with the exception of Hyla lanciformis. This species is not chlorotic. Conversely, the red blood cells of several other species did not hemolyze in their green plasma. From these observations it would appear that biliverdin is not necessarily responsible for hemolysis, or that it is effective only under certain conditions or in certain species.

Coagulation of red cell cytoplasm was another notable feature of some blood smears. The species of Atelopus, Hyla and Leptodactylus which demonstrated this character have little or no tendency toward chlorosis. Within this group, the small species of Hyla, such as Hyla boesemani, Hyla leucophyllata, and Hyla minuta, as well as the chlorotic Sphaenorhynchus aurantiacus are found in relatively exposed breeding habitats. Since it has been shown that exposure to a temperature of 45° C. causes coagulation of frog erythrocyte cytoplasm in vitro, it appears that high environmental temperatures are responsible for this condition under natural conditions. Tadpoles of Hyla dominicensis have been observed at 40° C. in nature for

several hours. In addition, Bokermann's (1965) observation that Osteocephalus taurinus was subjected to air temperature of 46° C. shows that the effect of heat is a likely cause of protein coagulation and hemolysis in Neotropical anurans. Heating of human blood to 40° C. for extended periods decreases solubility of hemoglobin (Goldberg, 1958); heating to 50° C. for ten minutes or more causes hemolysis (Kimber and Lander, 1964). Cloudsley-Thompson (1965) found that heat death of tropical lizards was due to physiological oxygen deficiency and another factor which operated simultaneously, perhaps protein coagulation. Thus, it appears that the oxygen transport system reaches the limit of its capacity at about the same temperature which causes the degeneration of the system because of coagulation of hemoglobin and hemolysis.

The blood cells of Hyla punctata are quite variable in size and shape, and have basophilic cytoplasm. In fact, these cells appear to be immature and closely resemble those of erythropoietic tissue. It seems that these immature cells have been released into the circulating blood to compensate for cell loss, probably through hemolysis. Erythrocytes of Phyllomedusa hypochondrialis also appear basophilic, but are relatively uniform in size and shape. It is possible that these cells are immature, but it seems more likely that the basophilia is tied in with the presumably low metabolic rate of these slow moving creatures.

Additional evidence of hemolysis is suggested by the presence of cell fragments in the liver circulation of Pseudis paradoxus.

Since most chlorotic species do not have coagulated cytoplasm in the erythrocytes present, and since most of the species with coagulated

cytoplasm are not chlorotic, these two characters appear to be mutually exclusive to a large extent. Thus the possibility arises that the erythrocytes of the chlorotic species are more susceptible to lysis (and perhaps coagulation as well) than are the red cells of non-chlorotic frogs. One may hypothesize that high temperatures cause cytoplasmic coagulation in both types of frogs, but that hemolysis, leading to biliverdin formulation, occurs only in the chlorotic species.

Among frogs, two other types of increased hemolysis are likely. Varela and Sellares (1938) found that Bufo arenarum in Brazil has a peak number of red blood cells (one million per cubic millimeter) in July and August with a rapid decrease in blood count in October, at the end of the breeding season. During the present work, frogs studied in Surinam in July (at the end of the breeding season) sometimes demonstrated green plasma but did not have green bones. The assumption was made that the high concentration of biliverdin was of a transitory nature and did not last long enough to cause staining of relatively permanent tissues such as bone. Similarly, the presence of green bones in animals without green plasma gives additional evidence of seasonal peaks of biliverdin concentration. Bone cross-sections showing alternate regions of dark and light green color in Lysapsus mantidactylus (Barrio, 1965[a]) and in Osteocephalus taurinus also indicate periodic hemolysis. One may suggest that hemolysis at the end of the breeding season is due to hormonal changes or to effects of higher temperatures encountered during the breeding season, or both.

Another type of large-scale hemolysis found among anurans occurs at metamorphosis. McCutcheon (1936) has shown that larval hemoglobin of Rana catesbeiana is different from that of the adult. Herner and Frieden (1961) have shown that the hemoglobins of larval Xenopus laevis, Rana heckscheri and Rana catesbeiana are replaced by adult hemoglobins during the course of metamorphosis. It seems likely that this is the explanation for the appearance of biliverdin during metamorphosis of most West Indian hylids. A similar change from fetal to adult hemoglobin in man is partly responsible for neonatal jaundice.

Phytohemagglutinins may be used to hemolyze erythrocytes and induce division of leukocytes in frog blood. However, the effects of these water-soluble plant derivatives in vivo are poorly known (Boyd, 1963).

Deficiency of vitamin E may result in changes to the cell membrane of red blood cells, causing them to hemolyze more readily. This may be a factor in frogs which appear to have more fragile erythrocytes.

A possibility which has been considered unlikely is that hemolysis may be a result of the effect of ultraviolet light upon hemoglobin (Lemberg and Legge, 1949). In view of the fact that blue light may be used to reduce circulating bilirubin in infants (Broughton et al., 1965), it is conceivable that the high incidences of ultraviolet light in tropical areas may have an effect on circulating red cells.

Increase in circulating bile salts as a result of defective liver function may be a cause of hemolysis (Grodins, Berman and Ivy, 1941). These authors found the apocholate and deoxycholate salts to be the most

toxic on the basis of degree of hemolysis produced. Reeder (1964) has recently summarized the meager knowledge concerning amphibian bile salts, none of which refers to species known to have biliverdin in tissues.

Among West Indian hylids, only Hyla marianae has orange bones during metamorphosis. The reasons for this species being different from the others are unknown, but speculation is possible. The extract of Hyla marianae skin contains at least one light orange pigment. Although the pigment was not identified, it shares spectral characteristics (Figure 6) with formyl derivatives of folic acid (Stokstad and Koch, 1967). Coenzymes containing folic acid are important in transfer of certain one-carbon groups, oxidation-reduction reactions, synthesis of purines and some pyrimidines, and in amino acid metabolism, including the transformation of phenylalanine to tyrosine. Thus, there seems to be the possibility that this species has a rich supply of folic acid or similar substance which could allow the liver cells to divide rapidly (because of readily available purines and pyrimidines for DNA synthesis) in order to excrete an increased amount of biliverdin; produce more melanin (because of availability of tyrosine) than pale, green forms such as Hyla wilderi; and have sufficient folic acid left that it could be used to eliminate formyl groups (perhaps from enzymatic biliverdin formation) by way of the skin. An excess of this vitamin might account for the relatively large size of these tadpoles at metamorphosis, as well as their aggressiveness. The source of folic acid is not readily apparent, but might be the leaves of the small bromeliads in which this species was found. An alternate

hypothesis for lack of green pigment in this species is that the liver of this relatively large tadpole is better developed at metamorphosis, and therefore can cope with the heavy load thrust upon it at metamorphosis, including an increase of biliverdin.

Experiments reported on earlier in this paper indicate that hemolysis alone is not likely to result in high concentrations of biliverdin in the tissues. One cubic centimeter of a 1 per cent hemoglobin solution injected into a 1 gram specimen of Hyla cinerea produced no noticeable effect. This amount of hemoglobin (0.01 gram) would result in the production of 0.46 milligrams of biliverdin in a rather short period (hours or a few days). Apparently, it is excreted without difficulty and without green pigmentation developing in the tissues. If we estimate that 5 per cent of such a frog consists of blood, that one-fifth of that consists of red blood cells, and that hemoglobin constitutes one-half of the red cell weight, we would calculate that it contains 0.005 grams of hemoglobin. Comparison of these two sets of calculations would show that the frog can degrade an amount of hemoglobin equivalent to twice that contained within its body, in a few days, without accumulating biliverdin in the tissues.

Bile pigment formation in frogs has not been studied in regard to the source of biliverdin. It is presumed that hemoglobin is the primary source of bile pigment, and that other hemoproteins yield a minor fraction.

Defective Transport of Bile Pigment into the Liver Cell

The importance of this factor in the accumulation of biliverdin in frogs is not known.

Defective Bile Pigment Conjugation

Although it is possible for small amounts of anuran biliverdin to be enzymatically conjugated as a glucuronide (Noir, Rodriguez Garay and Royer, 1965), Barrio (1965[a]) found that the biliverdin of frogs reacts as if unconjugated. Thus, a deficiency or inhibition of this conjugating enzyme system probably would not be important in the green pigmented frogs.

Disturbed Bile Pigment Excretion

Excretion of bile pigment may be blocked either within the liver or in the ducts leading away from the liver.

Intrahepatic cholestasis may be a constitutional disease which is often familial. Dubin-Johnson syndrome (Dubin, 1958) is a chronic, intermittent, benign form of jaundice in which the liver is often greenish black and microscopically shows much pigment which is probably lipofuscin. In these characteristics it resembles the situation seen in Pseudis paradoxus, which has a black liver in the adult.

Drugs can cause an intrahepatic blockage of bile due to their effects upon hepatic cells. Important among these are certain steroid hormones and chlorpromazine, as well as others (Sherlock, 1964). It is possible that frog liver is affected by hormones since Bachmann, Goin and Goin (1966) have shown that there is an increase in polyploidy of frog liver cells with the onset of the breeding season. Steroid hormones are known to increase polyploidy in other forms. Thus, it seems possible that tropical frogs may have an extended breeding season which affects the liver adversely. Evidence of this was seen in the liver of Hyla maxima

which showed development of fatty areas, which can be caused by extended use of steroid hormones or deficient diet.

Destruction of liver cells by chemicals such as carbon tetrachloride or situations accompanying acute virus hepatitis may result in intrahepatic obstruction (Williams, 1965). Tannic acid is a chemical which causes damage to the liver in mammals (Arhelger, Broom and Boler, 1965) and may be a cause of liver injury to tadpoles which live in waters with significant concentration of this chemical.

Allen, Carstens and Olson (1967) studied veno-occlusive disease in monkeys. A single dose of monocrotaline causes necrosis of liver cells and partial blockage of the veins within the liver. This drug is believed to be the ingredient of bush teas which cause veno-occlusive disease in Jamaica. Venous occlusion was observed in Hyla geographica in Surinam. It may have been induced by a plant extract.

Another factor which has an effect upon bile flow is the concentration of environmental oxygen. Anesthetized dogs which breathed air with 15 per cent oxygen for thirty to forty-five minutes had the bile flow reduced by 16 to 50 per cent. There was also a reduction of urine formation (Schnedorf and Orr, 1941). The tadpoles studied lived under conditions of low oxygen tension, but all appeared to utilize atmospheric oxygen when placed under stress. However, the conditions of low oxygen tension and high temperature to which Hyla dominicensis tadpoles were subjected, would appear to push the limit in this direction. Also, a high respiratory rate, such as appears to be present in tadpoles of Hyla heilprini may result in a relative oxygen lack that would reduce bile flow and thus

cause their extreme green pigmentation. It should be noted that these tadpoles were in the process of metamorphosis and may have had an excessively high respiratory rate for this reason.

The very low metabolic rate of Pseudis tadpoles may be an adaptation to low oxygen concentrations within their environment or be correlated with their large size, but they still develop green tissues.

Desiccation could well be a factor contributing to reducing the flow of bile through the liver. With less internal water for physiological purposes, secretion and excretion of bile may be impaired, so that bile pigments would occur in the blood. Since the tree frogs, in their arboreal habitat, appear to be more susceptible to chlorosis than do the ground frogs, one might suppose that their more exposed position is the reason. Desiccation is also indicated by the concentrated condition of the bile.

Obstruction of the extra-hepatic bile ducts by gall stones, parasites or tumors was not observed in the frogs studied. One specimen did have an inflamed duodenum which may have reduced bile flow into that portion of the intestine. In most specimens, the gall bladder was filled with green bile and the feces were dark, indicating that bile was passing out of the liver, and into the intestine, respectively.

Advantages of the accumulation of green pigment in skin and other organs seem to lie largely in the realm of protective coloration. In a number of species, the green color is rarely visible externally, but in some of the smaller species--Hyla wilderi, and species of Centrolenella and Sphaenorhynchus--the green pigment is quite apparent and blends well with the green foliage on which these forms are found. This green

pigmentation of the skin appears to have advantages over the green color produced by the physical arrangement of different types of chromatophores. It appears to be long lasting and apparently does not change according to light, temperature, or hormonal conditions. As long as the frog remains associated with green vegetation, this should not be particularly disadvantageous. Perhaps the efficiency of energy utilization in the pigmented forms provides a greater advantage. Since the green pigmentation results from diffusion of an apparently harmless waste product into skin and other organs, little or no metabolic energy is required for the coloring process. In addition, chromatophores may be reduced without selective disadvantages, thus decreasing the nutrients and energy needed to maintain chromatophores.

Disadvantages where chlorosis is the normal condition are not readily apparent, but Lutz and Lutz (1938) stated that Sphaenorhynchus orophilus loses its green color when not in good health, a situation opposite to that which would be expected if the green indicated a diseased condition.

CONCLUSIONS

1. The green coloration of bones, soft tissues, and plasma of Neotropical anurans has been shown to be due to the presence of a green pigment.

2. Identification of the pigment as biliverdin has been confirmed.

3. The high concentrations of biliverdin have been found only in frogs which inhabit the Neotropical Region. It appears that this phenomenon is restricted to tropical tree frogs and to the aquatic frogs of the family Pseudidae.

4. For the first time, chlorosis has been found in tadpoles of several species.

5. Incidence of the pigmentation does not appear to be restricted to either sex or to any particular stage of the development, although it does not appear until metamorphosis in the tree frogs.

6. There are indications that the pigment concentration is higher during metamorphosis and at the end of the breeding season, than at other times.

7. While it appears that the green pigmentation is restricted to three families of tree frogs and the Pseudidae, this is not considered to be an indication of relationship between the Pseudidae and the tree frogs.

8. In different taxa, the presence of green pigment may vary at familial, generic, specific, subspecific or individual levels.

9. Frogs and tadpoles with green pigmentation have been collected from habitats which differ widely in concentrations of oxygen, dissolved salts, iron and phosphate, in addition to temperature, insolation, altitude and presence or absence of water currents. These organisms also differ widely in their food, feeding habits and methods of gaseous exchange.

10. The actual cause of the presence of high concentrations of biliverdin is not known. However, it appears that the source of the pigment is hemoglobin from red blood cells which are hemolyzed.

11. It seems likely that a combination of high temperatures and fragile erythrocyte membranes may cause hemolysis in some breeding adults. Hormonal changes at the end of the breeding season and during metamorphosis may also account for some types of hemolysis.

12. While hemolysis apparently is the source of pigment, it is unlikely that this alone could cause the green coloration of tissues. Since hemolysis probably occurs in these species during metamorphosis and at the end of the breeding season, both of which are times when the liver has great stress from other activities, it appears that the liver cells are unable to handle the increased bile pigment at this time. Since the biliverdin appears to be relatively non-toxic, its elimination presumably has a low priority.

13. Some species evidently have taken evolutionary advantage of the green bile pigment accumulation by using it as protective coloration.

14. In general, it seems that the green pigmentation is due to increased hemolysis coupled with a decreased ability of the liver cells to excrete this bile pigment, so that it accumulates in plasma, then stains the proteins of soft tissues and finally those of bone. High environmental temperatures and hormonal changes are considered the most likely causes of hemolysis, and perhaps impairment of liver function as well.

APPENDICES

Tables 1 - 10

Figures 1 - 20

TABLE 1. OCCURRENCE OF GREEN COLOR IN TISSUES AND FLUIDS OF ADULT ANURANS

P - Green color present A - Green color absent

FAMILY AND SPECIES	BONE	SOFT TISSUES	PLASMA	BILE	SOURCE
Rhinophrynidae					
Rhinophrynus dorsalis(1)	A	A	A	-	
Ranidae					
Phyllobates sp.(1)	A	A	A		
Rhacophoridae					
Hyperolius sp.	-	P(eggs)	-	-	M. Stewart (Pers. Comm.)
Microhylidae					
Elachistocleis ovale(3)	A	A	A	P	
Pseudidae					
Lysapsus limellum laevis	P	-	-	-	Parker, 1935
Lysapsus limellum limellum	-	-	P	-	Barrio, 1965a
Lysapsus mantidactylus	P	-	P	-	Barrio, 1965a
Pseudis minuta	P	-	-	-	Peters, 1873
Pseudis paradoxus paradoxus	P	P	A	P	
Pseudis paradoxus paradoxus(3)	-	A	A	P	
Pseudis paradoxus platensis	P	-	P	-	Barrio, 1965a Camerano, 1879
Bufonidae					
Bufo typhonius(3)	A	A	A	P	

TABLE 1 (Continued)

FAMILY AND SPECIES	BONE	SOFT TISSUES	PLASMA	BILE	SOURCE
Atelopodidae					
Atelopus sp.	A	A	A	P	
Hylidae					
Anotheca coronata	P	-	-	-	C. J. Goin (Pers. Comm.)
Hyla albofrenata	P	-	-	-	Cochran, 1955
Hyla albomarginata	P	-	-	-	Cochran, 1955
Hyla berthae	-	-	A	-	Barrio, 1965a
Hyla brunnea	A	A	A	P	
Hyla boesemani(1)	P	P	-	P	
Hyla calcarata(1)	P	P	P	P	
Hyla crepitans(2)	P	P	P	P	
Hyla cuspidata	P	-	-	-	B. Lutz, 1954
Hyla dominicensis	P or A	-	-	-	Lynn, 1958
Hyla egleri(2)	A	A	A	P	
Hyla geographica(1)	P	A	A	P	
Hyla heilprini(1)	P	P	P	P	
Hyla lanciformis(4)	A	A	A	P	
Hyla langsdorffi	P	P	-	-	B. Lutz, 1954
Hyla leucophyllata(1)	A	A	A	P	
Hyla lichenata(1)	P	-	-	-	
Hyla marianae(7)	A	A	A	-	
Hyla maxima(3)	P	P	P	P	
Hyla misera(1)	A	A	P	P	

TABLE 1 (Continued)

FAMILY AND SPECIES	BONE	SOFT TISSUES	PLASMA	BILE	SOURCE
Hyla nasica	-	-	P	-	Barrio, 1965a
Hyla pulchella andina	-	-	P	-	Barrio, 1965a
Hyla pulchella riojana	-	-	P	-	Barrio, 1965a
Hyla pulchella cordobae	-	-	P	-	Barrio, 1965a
Hyla pulchella pulchella	-	-	A	-	Barrio, 1965a
Hyla pulchella prasina	-	-	A	-	Barrio, 1965a
Hyla phrynoderma	-	-	A	-	Barrio, 1965a
Hyla punctata(2)	P	P	P	P	
Hyla punctata	P	-	P	-	Barrio, 1965a
Hyla raniceps	-	-	A	-	Barrio, 1965a
Hyla rubra(4)	A	A	A	P	
Hyla septentrionalis	P or A	A	P or A	P	
Hyla siemersi	-	-	P	-	Barrio, 1965a
Hyla squalirostris	-	-	A	-	Barrio, 1965a
Hyla trachytorax	-	-	A	-	Barrio, 1965a
Hyla vasta	P or A	-	-	P	
Hyla wavrini	P	-	-	-	Rivero, 1961
Hyla wilderi(4)	P	P	-	-	
Osteocephalus taurinus(2)	P	P or A	P or A	P	
Phyllomedusa bicolor(1)	A	A	A		
Phyllomedusa helenae	-	green eggs	-	-	Starrett, 1960
Phyllomedusa hypochondrialis(5)	A	A	A	P	

TABLE 1 (Continued)

FAMILY AND SPECIES	BONE	SOFT TISSUES	PLASMA	BILE	SOURCE
Phyllomedusa sauvagii	-	-	A	-	Barrio, 1965a
Phrynohyas venulosa	P	-	P	-	Barrio, 1965a
Phrynohyas venulosa(4)	P	P	P	P	
Sphaenorhynchus aurantiacus(5)	P	P	P	P	
Sphaenorhynchus dorisae	P	-	-	-	Goin, 1957
Sphaenorhynchus habrus	P	-	-	-	Goin, 1957
Sphaenorhynchus orophilus	P	-	-	-	Goin, 1957
Trachycephalus nigromaculatus	P	-	-	-	Cochran, 1955
Leptodactylidae					
Eleutherodactylus (several species)	A	-	-	P	
Leptodactylus sp.(2)	A	A	A	P	
Leptodactylus pentadactylus(1)	A	A	A	-	
Centrolenidae					
Centrolenella albomaculata	P	-	-	-	Savage, 1967
Centrolenella fleischmanni	A	-	-	-	Dunn, 1931
Centrolenella fleischmanni	-	green eggs	-	-	Starrett, 1960
Centrolenella granulosa	P	-	-	-	Savage, 1967
Centrolenella ilex	P	-	-	-	Savage, 1967
Centrolenella prosoblepon	P	P	-	-	Dunn, 1931

TABLE 1 (Continued)

FAMILY AND SPECIES	BONE	SOFT TISSUES	PLASMA	BILE	SOURCE
Centrolenella pulveratum	P	P	-	-	Dunn, 1931
Centrolenella reticulata	-	green eggs	-	-	Starrett, 1960
Centrolenella spinosa	P	-	-	-	Savage, 1967
Centrolenella vanzolinii	P	-	-	-	Barrio, 1965a

TABLE 2. OCCURRENCE OF GREEN PIGMENT AMONG METAMORPHOSING NEOTROPICAL ANURAN TADPOLES

SPECIES	BONE	SOFT TISSUES	PLASMA	BILE
PSEUDIDAE				
Pseudis paradoxus	P	P	P	P
HYLIDAE				
Hyla brunnea	P	P	-	P
Hyla dominicensis	P	P	-	P
Hyla heilprini	P	P	P	P
Hyla lichenata	P	P	-	-
Hyla marianae	A	A	-	-
Hyla septentrionalis	P	P	-	-
Hyla vasta	P	P	-	-

TABLE 3. LOCALITY DATA OF TADPOLE HABITATS

LOCATION AND HABITAT DATA	DATE	ELEVATION (Meters)	AIR TEMPERATURE
Bromeliads of Jamaica - habitat of Jamaican tree frogs and tadpoles			
1. Rose Valley	25 X 1965	490	-
	14 V 1966	490	28.5-33.5
	10 VIII 1966	490	26.4-31.2
2. 10 km NW of Troy	16 V 1966	750	26.0-27.5
3. 6 km S of Mandeville	8 VIII 1966	550	24.7-28.2
4. Point Hill	17 V 1966	600	25.0
5. Cavalier District, St. Andrew	27 X 1965	600	26.7
6. 1.6 km W of Wakefield	27 X 1965	350	28
7. 6.4 km NW of Juan de Bolas	27 X 1965	600	24
Bromeliad of Puerto Rico - habitat of no known tadpoles			
8. El Yunke - Biological Station	6 V 1966	800	-
Ponds and standing waters of Haiti - habitats of *Hyla dominicensis*			

TABLE 3 (Continued)

LOCATION AND HABITAT DATA	DATE	ELEVATION (Meters)	AIR TEMPERATURE
9. 8 km W of Port-au-Prince (flooded wiregrass pasture)	12 V 1966	1	30.2
10. 32 km W of Port-au-Prince (wheel ruts)	4 VIII 1966	10	33.2-35.2
11. 4.8 km W of Port-au-Prince	10 V 1966	200	26.8
(shaded pool in stream bed)	12 V 1966	200	27.2
Ponds and standing waters of Grand Cayman, B.W.I. - habitats of Hyla septentrionalis			
12. 3.2 km N of Georgetown (pond)	29 X 1965	4	31.7
13. Hell (roadside ditch)	29 X 1965	4	30.5
14. South Sound (two mangrove swamps)	29 X 1965	2	29.5-30.5
15. South Sound (four woodland ponds)	29 X 1965	2	30.5-32.2
16. 4.8 km E of Georgetown (cow well)	29 X 1965	2	29.5
17. 8 km E of Georgetown (cistern)	29 X 1965	2	28.3
18. Boddentown to East End (three ponds)	29 X 1965	3	27.2-28.9
19. North Side - (woodland pond)	30 X 1965	3	27.2
20. North Side - (large open pond)	30 X 1965	3	27.7

TABLE 3 (Continued)

LOCATION AND HABITAT DATA	DATE	ELEVATION (Meters)	AIR TEMPERATURE
Open pool of Florida, U.S.A. - habitat of *Hyla septentrionalis*			
21. SW Miami (pool of Serpentarium)	25 VI 1966	10	31.2-32.2
Pool of Trinidad - habitat of *Phyllomedusa bicolor* tadpoles			
22. Simla Biological Station	29 VII 1966		30.2
Ponds and standing waters of Surinam			
23. Albina Road at Commewijne River (roadside ditch)	13 VII 1966	10	29.0
24. Domberg (roadside ditch)	17 VII 1966	5	32.8
25. 28 km S of Paramaribo (ditch)	17 VII 1966	60	27.5
26. Overtoom Road (pond and pool)	25 VII 1966	60	29.0-29.3
27. Onverwacht (flood meadow)	25 VII 1966	60	27.0-30.2
Streams of Surinam			
28. Brownsberg - Princess Irene Falls (habitat of *Hyla maxima*)	20 VII 1966 21 VII 1966	350 350	24.7-25.1 24.5-25.0
29. Base of Brownsberg - Brown's Kreek	21 VII 1966	200	27.8

TABLE 3 (Continued)

LOCATION AND HABITAT DATA	DATE	ELEVATION (Meters)	AIR TEMPERATURE
Streams of Haiti			
30. Furcy (habitat of Hyla vasta and Hyla heilprini)	10-12 V 1966 2 VIII 1966	1350 1350	20.5-29.5 25.4-26.0
31. Riviere Froid, 4.8 km W of Port-au-Prince	10 V 1966	150	26.7
Streams of Jamaica - habitat of Bufo marinus			
32. Vicinity of Troja (two streams)	9 VIII 1966	450	28.7-30.0
33. 4.8 km W of Bog Walk (irrigation ditch)	9 VIII 1966	350	28.2
34. Vicinity of Kellitts (two streams)	17 V 1966	700	24.0-25.3
Streams of Puerto Rico - habitat of Leptodactylus albilabrus			
35. El Yunke - roadside stream	6 V 1966	750	22.2

TABLE 4. WATER CHARACTERISTICS OF TADPOLE HABITATS

LOCATION, HABITAT AND DATE	TEMPERATURE	OXYGEN (ppm)	HARDNESS (ppm)	pH
Bromeliads of Jamaica - habitat of Jamaican tree frogs and tadpoles				
1. Rose Valley - (25 X, in sun)	24.0-28.0(6)	-	-	5-7
(25 X, in shade)	23.0-26.0(17)	-	-	5.5-7
(14 V, in sun)	25.5-28.5(4)	1.4-2.4(4)	20	5
(14 V, in shade)	25.5-25.7(3)	1.2-2.4(3)	40	-
(10 VIII, in sun)	27.2	2.7	-	5.5
(10 VIII, in shade)	24.8-25.3(3)	3.0-3.2(3)	-	6.5-7.0
2. 10 km NW of Troy - (16 V)	24.0-26.1(3)	1.6-1.9(3)	50	5.5
3. 6 km S of Mandeville - (8 VIII)	22.9-23.7(4)	2.3-6.6(4)	-	5.6
4. Point Hill - (17 V)	22.5	1.1	-	5.5
5. Cavalier District - (27 X)	25.0-27.0(8)	-	8-40	5-6
6. 1.6 km W of Wakefield - (27 X)	25.0-25.5(3)	-	10	5-5.5
7. 6.4 km NW of Juan de Bolas (27 X)				
(without dust on leaves)	24.0	-	16-30(3)	5.5-6.5
(with dust on leaves)	24.5	-	70-90(3)	7.0

TABLE 4 (Continued)

LOCATION, HABITAT AND DATE	TEMPERATURE	OXYGEN (ppm)	HARDNESS (ppm)	pH
Bromeliad of Puerto Rico - habitat of no known tadpoles				
8. El Yunke Biological Station (6 V)	21.0	3.1	-	5.2
Ponds and standing waters of Haiti - habitats of Hyla dominicensis				
9. 8 km W of Port-au-Prince (12 V)	32.0	0.7	480	7.5
10. 32 km W of Port-au-Prince (4 VIII)	39.2-40.2(2)	1.4-8.0(2)	220(1)	7.5
11. 4.8 km W of Port-au-Prince (10 V)	25.1	2.2	200	-
(12 V)	24.6	1.7	200	7
Ponds and standing waters of Grand Cayman, B.W.I. - potential habitats of Hyla septentrionalis				
12. 3.2 km N of Georgetown (29 X)	32	-	64	7.0
13. Hell (29 X)	33.5	-	60	7.0
14. South Sound (29 X)	28-33(2)	-	84	7.5
16. 4.8 km E of Georgetown (29 X)	32.5	-	50	7.0
18. Boddentown to East End (29 X)	28-29(3)	-	60-90	7.0-7.5

TABLE 4 (Continued)

LOCATION, HABITAT AND DATE	TEMPERATURE	OXYGEN (ppm)	HARDNESS (ppm)	pH
Actual habitats of Hyla septentrionalis				
15. South Sound (29 X)	29.0-34.0(4)	-	106-134	7.5
17. 8 km E of Georgetown (29 X)	25	-	60	7.5
19. North Side (30 X)	27	-	64	7.0
20. North Side (30 X)	26	-	70	7.0
Open pool of Florida, U.S.A. - habitat of Hyla septentrionalis				
21. SW Miami (25 VI)	32.9-33.9(2)	8.1-9.2(2)	-	-
Pool of Trinidad - habitat of Phyllomedusa bicolor				
22. Simla Biological Station	28.2	10.1	-	-
Ponds and standing waters of Surinam				
23. Albina Road at Commewijne River (13 VII)	27.8	3.4	-	-
24. Domberg (17 VII)	33.0	-	-	-
25. 28 km S of Paramaribo (17 VII) - habitat of Phrynohyas venulosa	25.0-29.4(2)	0.7	-	-
26. Overtoom Road (25 VII)	29.9-30.8(2)	6.5-7.1(2)	-	-

TABLE 4 (Continued)

LOCATION, HABITAT AND DATE	TEMPERATURE	OXYGEN (ppm)	HARDNESS (ppm)	pH
27. Onverwacht - habitat of *Hyla crepitans*, *Hyla punctata*, *Hyla minuta* and *Hyla boesemani* (25 VII-5PM)	30.9	3.4	-	-
(25 VII-7:40PM)	27.5	0.6	-	-
Streams of Surinam				
28. Princess Irene Falls - habitat of *Hyla maxima* (20-21 VII)	23.4-25.5(6)	7.8-8.6(6)	-	-
Rock basin (21 VII)	23.8-29.9	2.0-3.1	-	-
29. Brown's Kreek - habitat of fish (21 VII)	25.2	4.9	-	-
Streams of Haiti				
30. Furcy - habitat of *Hyla vasta* and *Hyla heilprini* (10-12 V)	19.5-24.5(14)	7.4-9.5(14)	40-90(3)	6-7(2)
(2 VIII)	20.1-25.2(5)	7.3-9.9(5)	60-90(5)	7
31. Riviere Froid - (10 V)	25.8	7.9	130	-
Streams of Jamaica				
32. Troja - habitat of *Bufo marinus* (9 VIII)	27.0-27.1(2)	7.9-8.0(2)	-	-

TABLE 4 (Continued)

LOCATION, HABITAT AND DATE	TEMPERATURE	OXYGEN(ppm)	HARDNESS(ppm)	pH
33. 4.8 km W of Bog Walk (9 VIII)	24.5	8.6	-	7
34. Kellitts (17 V)	22.0-22.5(2)	8.2-8.4(2)	80-130(2)	6.5-7.0
Streams of Puerto Rico				
35. El Yunke - habitat of Leptodactylus albilabrus (6 V)	21.0	3.1	20	6

TABLE 5. IRON AND PHOSPHATE CONCENTRATIONS (PARTS PER MILLION) IN TADPOLE HABITATS

HABITAT DATA AND LOCATION	IRON	PHOSPHATE	SPECIES OF TADPOLE PRESENT
Bromeliads of Jamaica			
1. Rose Valley	0.00	0.00	Hyla brunnea
	Trace	0.40	None
	0.05	2.2	None
	0.02	0.30	None
3. 6 km S of Mandeville	0.16	0.10	Hyla brunnea
	0.25	0.21	Hyla brunnea
	0.73	3.4	None
	0.30	2.7	None
Standing waters			
10. Wheel ruts 32 km W of	1.13	1.35	Hyla dominicensis
Port-au-Prince, Haiti	1.80	4.80	Hyla dominicensis
24. Domberg, Surinam	7.5	0.18	Pseudis paradoxus
25. 28 km S of Paramaribo	0.62	0.70	Phrynohyas venulosa
Surinam Stream habitats			
28. Princess Irene Falls	0.06	0.02	Hyla maxima (large)
Basin near the Falls	0.18	0.03	Hyla maxima (small)
30. Furcy, Haiti	0.10-0.26	0.35	Hyla heilprini, Hyla vasta
32. Troja, Jamaica	0.02-0.40	0.39-0.41	Bufo marinus
33. 4.8 km W of Bog Walk Jamaica	0.30	0.25	None

TABLE 6. BLOOD CHARACTERISTICS

FAMILY AND SPECIES	HEMOGLOBIN (Gram Per Cent)	RED CELL COUNT	HEMOGLOBIN PER CELL (Picograms)
Microhylidae			
Elachistocleis ovale	8.0	597,500	134
	13.0	805,000	161
	9.0	725,000	124
Pseudidae			
Pseudis paradoxus	7.0	887,500	79
	10.0	782,500	128
	9.0	767,500	117
	8.0	833,000	96
Buronidae			
Bufo typhonius	7.5	505,000	149
	6.0	460,000	130
Hylidae			
*Hyla calcarata	7.0	517,500	135
*Hyla crepitans	8.0	962,500	83
*Hyla geographica	8.0	682,500	117
*Hyla heilprini	11.4	400,000	285
Hyla lanciformis	5.5	320,000	172
	6.5	345,000	188
	7.5	477,500	157

TABLE 6 (Continued)

FAMILY AND SPECIES	HEMOGLOBIN (Gram Per Cent)	RED CELL COUNT	HEMOGLOBIN PER CELL (Picograms)
Hyla leucophyllata	7.0	460,000	152
Hyla marianae		543,000	
*Hyla maxima	14.5	967,500	150
	14.5	1,395,000	104
	7.5	512,500	146
*Hyla punctata	3.0	615,000	49
Hyla rubra	13.5	965,000	140
	7.5	675,000	111
	10.0	790,000	127
*Hyla vasta	11.0	742,500	148
*Hyla wilderi		212,000	
*Osteocephalus taurinus	6.5	660,000	98
*Phrynohyas venulosa	9.5	1,212,500	78
	4.5	950,000	47
	11.5	1,157,500	99
	16.0	1,378,000	116
Phyllomedusa bicolor	5.5	322,500	171
Phyllomedusa hypochondrialis	6.0	255,000	235
	6.5	287,500	226
	5.5	360,000	153
	7.0	478,000	146
*Sphaenorhynchus aurantiacus	7.5	585,000	128
	2.0	217,000	92

TABLE 6 (Continued)

FAMILY AND SPECIES	HEMOGLOBIN (Gram Per Cent)	RED CELL COUNT	HEMOGLOBIN PER CELL (Picograms)
Leptodactylidae			
Leptodactylus pentadactylus	7.0	467,500	150
Leptodactylus sp.	11.5	1,100,000	105
Leptodactylus sp.	6.5	850,000	76

* Chlorotic species

TABLE 7. AVERAGE DIMENSIONS OF FROG RED BLOOD CELLS

FAMILY AND SPECIES	AVERAGE CELL LENGTH ± 2 S.E.	AVERAGE CELL WIDTH ± 2 S.E.	PRODUCT OF CELL LENGTH x WIDTH
Pseudidae			
*Pseudis paradoxus	13.2 ± 0.73	12.0 ± 0.30	158.4
Bufonidae			
Bufo typhonius	16.3 ± 0.92	12.9 ± 0.39	210.3
Hylidae			
*Hyla boesemani	16.3 ± 0.63	11.9 ± 0.38	194.0
Hyla egleri	17.4 ± 1.48	12.6 ± 0.62	219.2
*Hyla geographica	15.8 ± 0.30	9.9 ± 1.74	156.4
Hyla leucophyllata	16.7 ± 0.61	13.4 ± 0.44	223.8
*Hyla maxima	18.3 ± 0.48	11.0 ± 0.33	201.3
Hyla minuta	15.1 ± 0.40	11.7 ± 0.43	176.7
*Hyla misera	14.0 ± 0.54	12.4 ± 0.48	173.6
*Hyla punctata	17.9 ± 1.05	12.8 ± 2.00	229.1
Hyla rubra	13.9 ± 0.43	11.4 ± 0.24	158.5
*Osteocephalus taurinus	13.1 ± 0.36	10.8 ± 0.40	141.5
Phyllomedusa bicolor	17.0 ± 0.73	13.7 ± 0.66	232.9
Phyllomedusa hypochondrialis	20.6 ± 0.89	14.5 ± 1.29	298.7
*Phrynohyas venulosa	12.8 ± 1.11	10.8 ± 0.27	138.2
*Sphaenorhynchus aurantiacus	21.6 ± 0.47	13.2 ± 0.39	285.1
Leptodactylidae			
Leptodactylus pentadactylus	17.2 ± 0.42	12.4 ± 0.33	213.3

Figures represent average for twenty-five cells from a single individual of each species.

* Chlorotic species

TABLE 8. RESPIRATORY RATES OF TADPOLES IN WARBURG RESPIROMETERS AT DIFFERENT TEMPERATURES

SPECIES AND GROUP	WEIGHT (mg)	OXYGEN UPTAKE (ul O_2/g/hr)					
		15°	20°	25°	30°	35°	40°
Group A							
Hyla brunnea	170			236	303	364	403
	275			264	399	483	319
	280			306	428	547	646
	286			253	387	460	390
Group B							
Hyla brunnea	152	41	108	133			
	252	113	147	186			
	260	123	152	225			
Group C							
Hyla septentrionalis	101			232	365	409	421
	152			239	352	536	574
	211			273	425	506	497
	219			260	317	395	371
Group D							
Hyla septentrionalis	179	108	131	273			
	190	82	88	138			
	201	113	132	217			
	191	106	117	257			

TABLE 9. TADPOLE RESPIRATORY RATES AT 25° C. (MEASURED BY JONES METHOD)

SPECIES	WEIGHT(mg)	OXYGEN UPTAKE (ul O_2/g/hr)
Hyla dominicensis	315	262
Hyla dominicensis	481	241
Hyla dominicensis	398	187
Hyla dominicensis	248	216
Hyla dominicensis	226	206
Hyla dominicensis	597	252
Hyla heilprini	1,203	255
Hyla heilprini	1,444	265
Hyla heilprini	1,420	277
Hyla vasta	476	203
Hyla vasta	706	248
Hyla vasta	370	329
Hyla vasta	83	663
Hyla vasta	84	669
Hyla vasta	170	560
Hyla maxima	69	739
Hyla maxima	70	714
Hyla maxima	78	756
Hyla maxima	84	678
Pseudis paradoxus	3,696	53
Pseudis paradoxus	5,948	31
Pseudis paradoxus	4,577	44

TABLE 9 (Continued)

SPECIES	WEIGHT(mg)	OXYGEN UPTAKE (ul O_2/g/hr)
Pseudis paradoxus	3,013	35
Pseudis paradoxus	6,782	41
Pseudis paradoxus	5,806	36

TABLE 10. FROGLET RESPIRATORY RATES MEASURED IN WARBURG RESPIROMETERS

SPECIES	WEIGHT(mg)	OXYGEN CONSUMED (25° C.) (ul O_2/g/hr)	OXYGEN CONSUMED (35° C.) (ul O_2/g/hr)
Hyla brunnea	112	167	482
	120	101	548
	128	279	611
	135	134	395
	215	152	406
	231	211	471
	237	159	459
	286	119	331
Four days beyond metamorphosis			
Hyla brunnea	225		549
Hyla lichenata	282		487

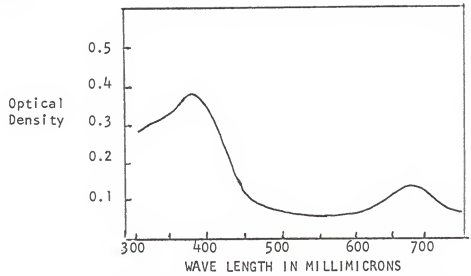

Figure 1. Absorption spectrum of biliverdin in 5 per cent hydrochloric acid - methanol solution.

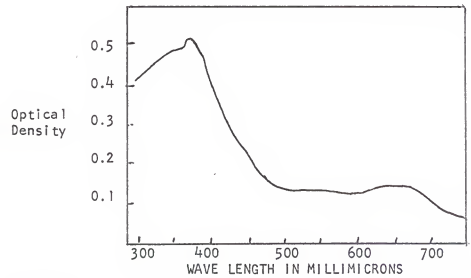

Figure 2. Absorption spectrum of liver extract from Hyla septentrionalis in aqueous solution.

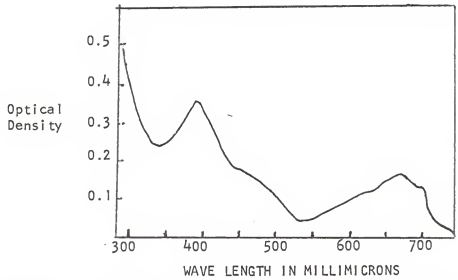

Figure 3. Absorption spectrum of lymph of Osteocephalus taurinus.

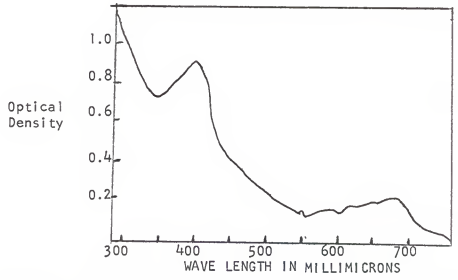

Figure 4. Absorption spectrum of coelomic fluid (bile) from Osteocephalus taurinus.

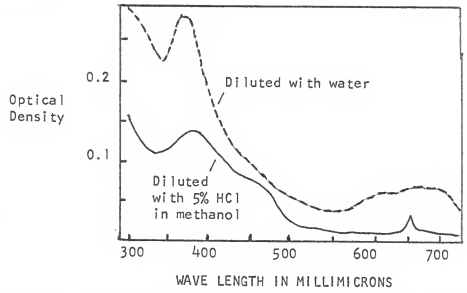

Figure 5. Absorption spectra of bile solutions of Siren lacertina.

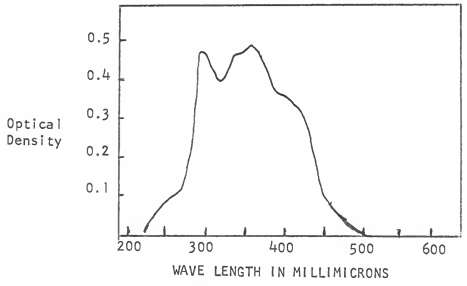

Figure 6. Absorption spectrum of pigment extracted from Hyla marianae in 70 per cent ethanol.

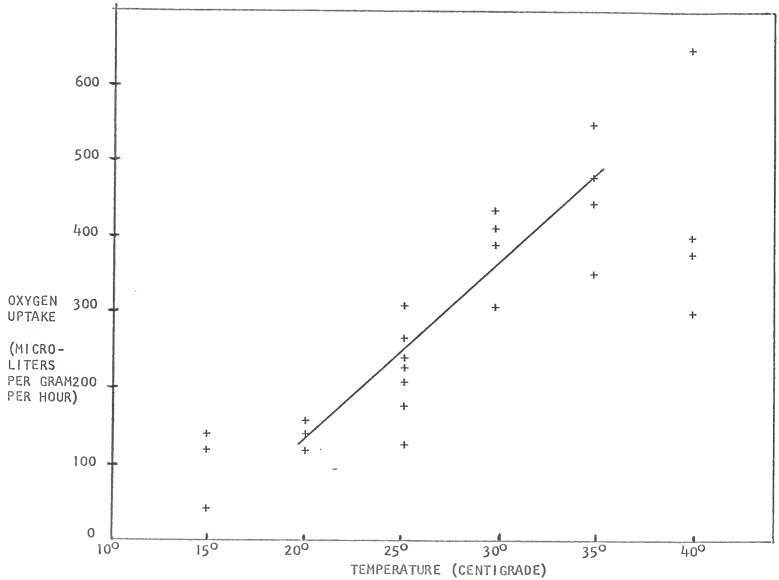

Figure 7. Respiratory rates of Hyla brunnea tadpoles at different temperatures (Table 8)

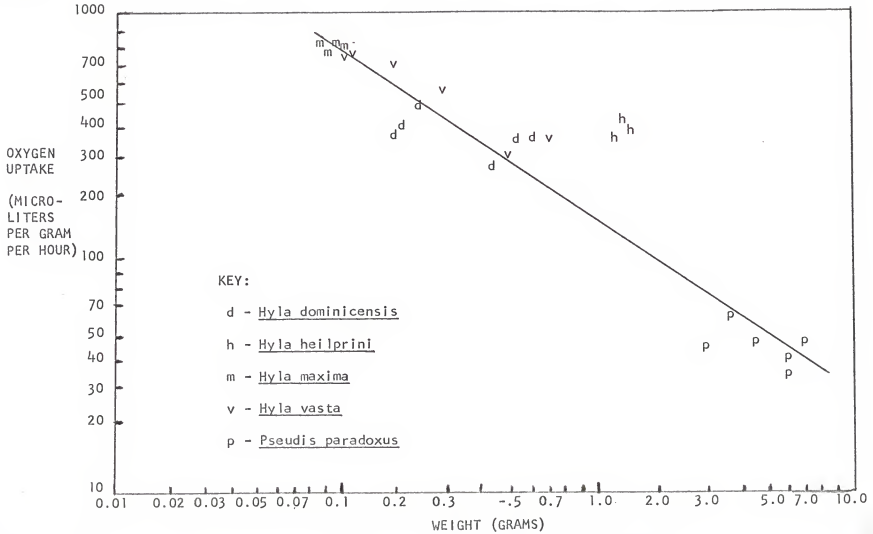

Figure 8. Respiratory rates of tadpoles at 25° C. (From Table 9)

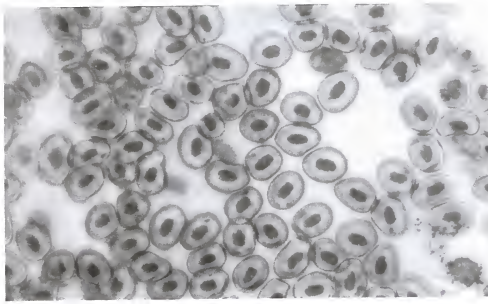

Figure 9. Normal red blood cells of Bufo terrestris four hours after collection. (400X)

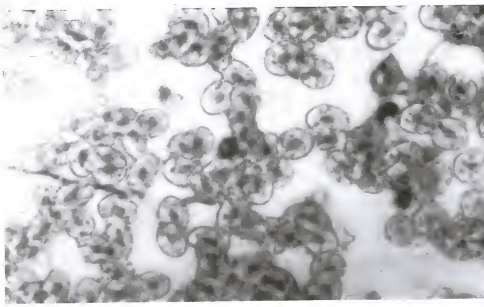

Figure 10. Red blood cells of the same individual as above, after four hours at 45° C. Note coagulated cytoplasm. (400X)

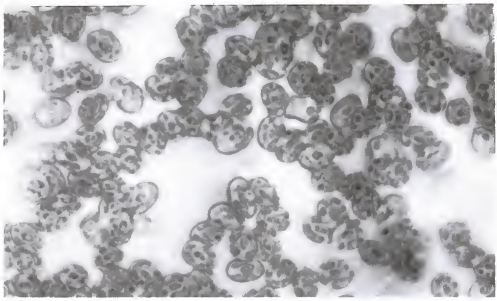

Figure 11. Red blood cells of Hyla squirella after four hours at 45° C. Note coagulated cytoplasm and tendency of cells to stick to each other. (400X)

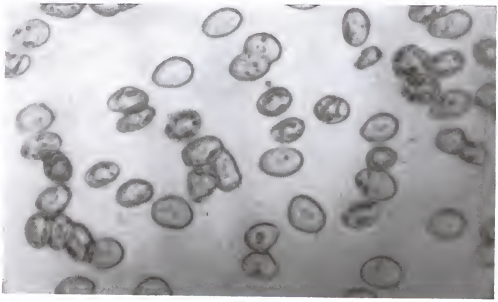

Figure 12. Untreated blood cells of Hyla leucophyllata. Note numerous erythrocytes with coagulated cytoplasm. (400X)

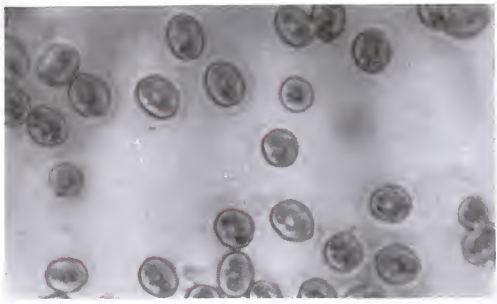

Figure 13. Stained smear of untreated blood of Bufo typhonius. Note coagulated cytoplasm. Green tissues are unknown in this species. (400X)

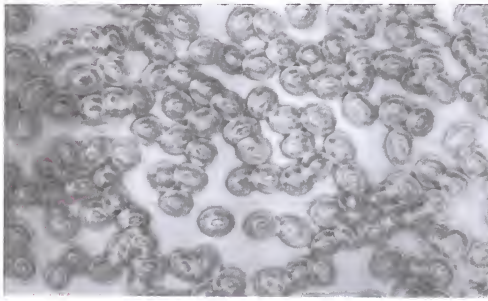

Figure 14. Stained smear of untreated blood of Phrynohyas venulosa. Note the clumped cytoplasm. (400X)

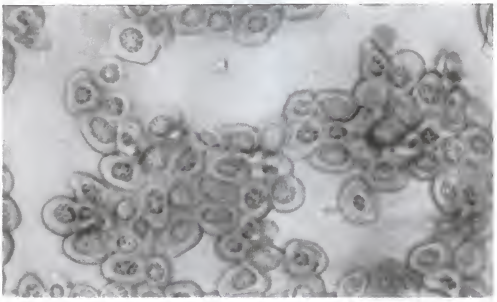

Figure 15. A stained smear of blood from Hyla punctata, a species with green tissues. Note that the majority of cells are immature, as indicated by their irregular shaped and large nuclei. (400X)

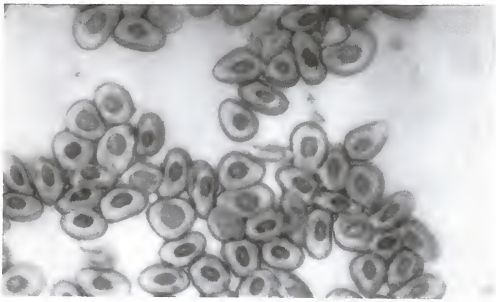

Figure 16. Stained smear of untreated Phyllomedusa bicolor blood. Cells were basophilic and perhaps immature in this frog which lacked green pigment. (400X)

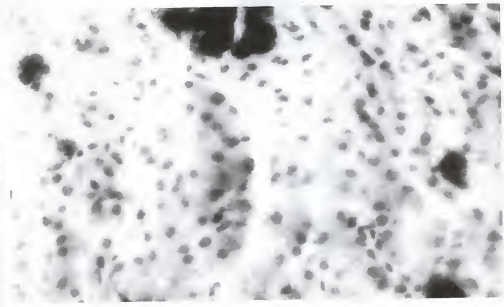

Figure 17. Stained section of apparently normal liver of Hyla rubra. (400X)

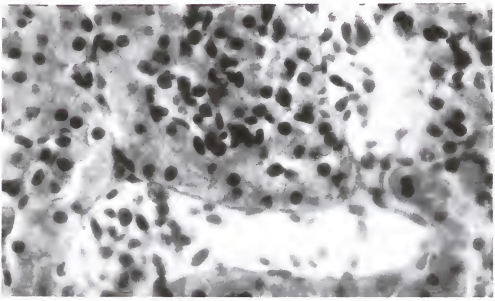

Figure 18. Stained section of Hyla punctata liver. Note the numerous nuclei, some of which appear pycnotic. (400X)

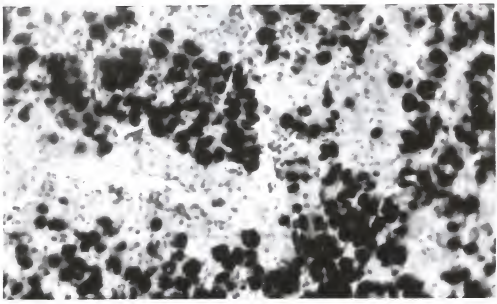

Figure 19. Stained section of liver from adult male Pseudis paradoxus. Note the large amount of pigment present. (200X)

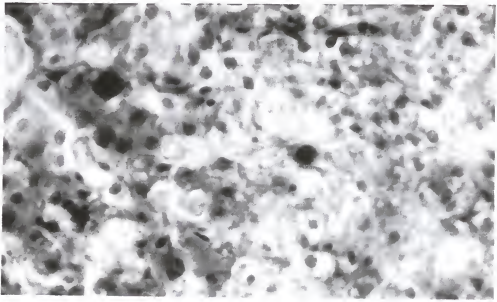

Figure 20. Stained section of liver from adult male Hyla maxima. Note degeneration of cytoplasm toward the lower right. (400X)

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BIOGRAPHICAL SKETCH

Duvall Albert Jones was born October 17, 1933, near Hurlock, Maryland. In June, 1951, he was graduated from Kenwood High School, Essex, Maryland. He received the degree of Bachelor of Arts from Western Maryland College in May, 1955. Following two years of military service, he enrolled in the Graduate School of the University of Maryland, from which he received the degree of Master of Science. Mr. Jones taught biology courses at Madison College, Harrisburg, Virginia, for two years. He accepted a position at Ferrum Junior College, Ferrum, Virginia, in September, 1962. He took a leave-of-absence from that position to enter the Graduate School of the University of Florida in September, 1963. Later, he accepted positions at West Liberty State College and Carnegie-Mellon University while continuing his graduate studies.

Duvall A. Jones is married to the former Dorothy Ann Paul. He is a member of the American Association for the Advancement of Science, the American Institute of Biological Sciences, the American Society of Zoologists, the American Society of Ichthyologists and Herpetologists, the Herpetologists' League and the Association of Southeastern Biologists.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Arts and Sciences and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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